Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

Organisers

Oscar Llorca Macromolecular complexes in DNA damage Response. (CNIO), Madrid, Spain

Rafael Fernández Leiro Genome Integrity and Structural Biology. (CNIO), Madrid, Spain

Laurence H. Pearl Genome Damage and Stability Centre-University of Sussex, UK

Titia Sixma Netherlands Cancer Institute, NKI, Netherlands

Speakers

James Berger Johns Hopkins School of Medicine, USA

Maria A. Blasco Spanish National Cancer Research Centre, Spain

Alessandro Costa, The Francis Crick Institute, UK

Patrick Cramer Max Planck Institute for Biophysical Chemistry, Germany

Aidan Doherty Genome Damage and Stability Centre, University of Sussex, UK

Daniel Durocher The Lunenfeld-Tanenbaum Research Institute, Canada

Karl-Peter Hopfner Gene Center Munich, Germany Meindert H. Lamers Leiden University Medical Center, Netherlands

Oscar Llorca Macromolecular complexes in DNA damage Response, CNIO, Spain

Juan Méndez DNA Replication Group, CNIO, Spain

Eva Nogales Lawrence Berkeley National Laboratory, Howard Hughes Medical Institute, USA

Lori Passmore Laboratory of Molecular Biology, MRC-LMB, UK

Laurence H. Pearl Genome Damage and Stability Centre-University of Sussex, UK Luca Pellegrini University of Cambridge, UK

Song Tan Penn State University, USA

Nicolas Thomä Friedrich Miescher Institute, Switzerland

Alessandro Vannini Institute of Cancer Research, ICR, UK

Roger Williams Laboratory of Molecular Biology, MRC-LMB, UK

Wei Yan<mark>g</mark> National Institutes of Health, NI</mark>H, USA

Xiaodong Zhang Imperial College London, UK



GOBIERNO DE ESPAÑA







🔨 "la Caixa" Banking Foundation

Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response



Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

#CFM_DNADamage
 @CNIO_Cancer
 @CaixaCiencia



GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES





* <u>"laCaixa" Banking Foundation</u>

Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response



Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

Summary

07 11	ORGANISERS & SPEAKERS PROGRAMME	
19 27 35 41 47 53 59	 SESSIONS S#1 Chromatin and chromatin complexes S#2a DNA Replication and replication stress S#2b DNA Replication and replication stress S#3a DNA transcription S#3b DNA transcription S#4a DNA damage and repair S#4b DNA damage and repair 	
67	ORGANISERS & SPEAKERS' BIOGRAPHIES	
91	POSTER SESSION	
135	Previous CNIO Frontiers Meetings and CNIO Cancer Conferences	





CNIO - LA CAIXA BANKING FOUNDATION FRONTIERS MEETINGS | 2019

Madrid 20 – 22 May 2019

Structural and molecular biology of the DNA damage response

Organisers and Speakers

Madrid 20 – 22 May 2019

Structural and molecular biology of the DNA damage response

Venue:

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid, Spain

Chairpersons and organising committee:

Oscar Llorca Spanish National Cancer Research Centre (CNIO), Madrid, Spain Rafael Fernández Leiro Spanish National Cancer Research Centre (CNIO), Madrid, Spain Laurence H. Pearl Genome Damage and Stability Centre-University of Sussex, Brighton, UK Titia Sixma Netherlands Cancer Institute, NKI, Amsterdam, Netherlands

Rationale:

DNA is continuously exposed to insults from endogenous and exogenous sources, and, therefore, maintenance of genome integrity is essential to all organisms. There are multiple pathways that ensure that the information contained in DNA is not damaged or lost. When these errors skip the control mechanisms or when other DNA damage type is present, multiple macromolecular complexes detect it and start the signalling cascades that lead to repair by different pathways depending on the lesion. All these pathways are safeguarding our genome and when they are de-regulated or they can't work efficiently, damage to DNA is accumulated. This, together with the instability of the genome, can promote the development of cancer and other diseases. This meeting will bring together research leaders in the field with a focus on the structural basis and molecular mechanisms.



Speakers

James Berger Johns Hopkins School of Medicine, USA

Maria A. Blasco Spanish National Cancer Research Centre, Spain

Alessandro Costa, The Francis Crick Institute, UK

Patrick Cramer Max Planck Institute for Biophysical Chemistry, Germany

Aidan Doherty Genome Damage and Stability Centre, University of Sussex, UK

Daniel Durocher The Lunenfeld-Tanenbaum Research Institute, Canada

Karl-Peter Hopfner Gene Center Munich, Germany Meindert H. Lamers Leiden University Medical Center, Netherlands

Oscar Llorca Macromolecular complexes in DNA damage Response, CNIO, Spain

Juan Méndez DNA Replication Group, CNIO, Spain

Eva Nogales Lawrence Berkeley National Laboratory, Howard Hughes Medical Institute, USA

Lori Passmore Laboratory of Molecular Biology, MRC-LMB, UK

Laurence H. Pearl Genome Damage and Stability Centre-University of Sussex, UK Luca Pellegrini University of Cambridge, UK

Song Tan Penn State University, USA

Nicolas Thomä Friedrich Miescher Institute, Switzerland

Alessandro Vannini Institute of Cancer Research, ICR, UK

Roger Williams Laboratory of Molecular Biology, MRC-LMB, UK

Wei Yang National Institutes of Health, NIH, USA

Xiaodong Zhang Imperial College London, UK





Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

Programme

Opening Remarks

Chair: Eva Nogales

09:15 - 09:45 *"Structural mechanism of the INO80 chromatin remodeller"* **Karl-Peter Hopfner** Gene Center Munich, Munich, Germany

S#1 Chromatin and chromatin complexes

Registration & welcome coffee for all the participants (hall)

- 09:45 10:15 *"Structural studies of chromatin complexes"* **Song Tan** Penn State University, Pennsylvania, US
- 10:15 10:45 *"Induction of telomeric DNA damage as a novel anti-cancer strategy"* **Maria A. Blasco** Spanish National Cancer Research Centre, Madrid, Spain
- 10:45 11:15 Coffee break (social room)

Monday May 20th, 2019

08:30 - 09:00

09:00 - 09:15

09:15 - 12:30

- 11:00 11:45 "DNA damage detection in nucleosomes involves DNA"
 Nicolas Thomä Friedrich Miescher Institute, Basel, Switzerland
- 11:45 12:15 *"Remodeling of RUVBL1-RUVBL2 ATPases by client proteins revealed by cryo-EM"* Oscar Llorca Spanish National Cancer Research Centre, Madrid, Spain
- 12:15 12:30 "Conformational Activation Promotes CRISPR-Cas12a Catalysis and Resetting of the Endonuclease Activity" Guillermo Montoya NNF-CPR, University of Copenhagen, Denmark
- 12:30 14:00 Lunch (cafeteria)

PROGRAMME

Monday May 20th, 2019

14:00 - 17:15 S#2a D Chair: F	NA Replication and replication stress Rafael Fernández-Leiro
14:00 - 14:30	<i>"Mechanisms for initiating DNA replication"</i> James Berger Johns Hopkins School of Medicine, Baltimore, US
14:30 - 15:00	"Operating principles and catalytic mechanism of DNA replisome" Wei Yang National Institutes of Health, NIH, Bethesda, US
15:00 - 15:30	<i>"Structural features of High Fidelity DNA Replication"</i> Meindert Lamers Leiden University, The Netherlands

- 15:30 16:00 Coffee break (social room)
 - 16:00 16:30 *"Molecular mechanisms of eukaryotic DNA replication and recombination"* Luca Pellegrini Cambridge University, Cambridge, UK
 - 16:30 17:00 *"Eukaryotic DNA replication studied by cryo-electron microscopy"* Alessandro Costa The Francis Crick Institute, London, UK
 - 17:00 17:15 "Cryo-EM structure of the human lagging strand DNA polymerase delta holoenzyme" Alfredo De Biasio University of Leicester, UK
- 17:15 18:45 Poster session, wine & cheese (Social Room)

Sponsored by



09:15 - 09:45 *"The 3Rs (Recruitment, Role and Regulation)* of PrimPol during DNA Replication in eukaryotic cells" **Aidan Doherty** Genome Damage and Stability Centre, University of Sussex, Brighton, UK

S#2b DNA Replication and replication stress

- 09:45 10:15 *"A role for PrimPol at highly cytotoxic DNA lesions"* Juan Mendez Spanish National Cancer Research Centre, Madrid, Spain
- 10:15 10:45 *"Structural studies of key components in DNA damage response"* Xiaodong Zhang Imperial College London, UK
- 10:45 11:15 Group picture (CNIO main door) & Coffee break
- 11:15 14:30 S#3a DNA transcription Chair: Lori Passmore
 - 11:15 11:45 *"TFIIH in transcription and DNA repair"* **Patrick Cramer** Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
 - 11:45 12:15 *"Unveiling RNA Polymerase III* (*extra*)*transcriptional complex with EM"* **Alessandro Vannini** Institute of Cancer Research, ICR, London, UK
 - 12:15 12:30 *"Structural basis of RNA polymerase I stalling at UV light-induced DNA damage"* **Carlos Fernández-Tornero** CIB-CSIC, Madrid, Spain

12:30 - 14:00 Lunch (cafeteria)

PROGRAMME

Tuesday May 21st, 2019

09:15 - 10:45

Tuesday May 21st, 2019

- 14:00 15:00 S#3b DNA transcription Chair: Wei Yang
 - 14:00 14:30 *"Molecular visualization of the transcription initiation"* **Eva Nogales** HHMI/University of California at Berkeley, Berkeley, US
 - 14:30 14:45 "The molecular mechanism of TFIIH recruitment to DNA damage-stalled RNA polymerase II during transcription-coupled DNA repair" Martijn Luijsterburg Leiden University Medical Center, The Netherlands
 - 14:45 15:00 *"Molecular basis of tRNA recognition by the Elongator complex"* **Maria I. Dauden** EMBL, Heidelberg, Germany
- 15:00 17:00 S#4a DNA damage and repair Chair: Wei Yang
 - 15:00 15:30 *"Phosphorylation dependent assembly of DNA damage response complexes"* Laurence H. Pearl Genome Damage and Stability Centre-University of Sussex, Brighton, UK
- 15:30 16:00 Coffee break (social room)
 - 16:00 16:30 *"Mechanistic insights into DNA repair using cryo-EM"* Lori Passmore MRC Laboratory of Molecular Biology, Cambridge, UK

Tuesday May 21st, 2019



"A conformationally versatile complex between Ctf18-RFC and DNA Pol ε is required for leading strand replication stress signaling" **Daniel Grabarczyk** University of Würzburg, Germany

16:45 - 18:30 Poster session – Wine & Cheese (social room)

Wednesday May 22nd, 2019

- 09:30 11:45 S#4b DNA damage and repair Chair: Oscar Llorca
 - 09:30 10:00 *"Charting the response to DNA damage using functional genomics"* **Daniel Durocher** The Lunenfeld-Tanenbaum Research Institute, Toronto, Canada
 - 10:00 10:30 *"Structural mechanisms of regulation of PIKK family members"* **Roger Williams** MRC Laboratory of Molecular Biology, Cambridge, UK
 - 10:30 10:45 *"Structural basis of allosteric regulation of Tel1/ATM kinase"* Gang Cai University of Science and Technology of China, China
- 10:45 11:15 Coffee break (social room certificates and invoices will be available at the reception desk)

Wednesday May 22nd, 2019

1

1

10:45 - 11:15 Coffee break (social room - certificates and invoices will be available at the reception desk)

1:15 - 11:30 SHORT TALK	"Binding and unwinding activities of human HELB unveiled by biochemical and biophysical methods" Fernando Moreno-Herrero CNB-CSIC, Madrid, Spain
1:30 - 11:45 SHORT TALK	<i>"CryoEM Structure of the XPF-ERCC1 Endonuclease Reveals an Auto-Inhibited Conformation"</i> Morgan Jones The Francis Crick Institute, London, UK

11:45 - 12:15 Prizes for best posters and best short talks

Poster awards sponsored by:



The world leader in serving science

Short talk awards sponsored by:



Closing remarks





Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response

Monday 20th

Session #1 Chromatin and chromatin complexes

Chairperson: Eva Nogales

Structural mechanism of the INO80 chromatin remodeller

Karl-Peter Hopfner

Gene Center Munich, Munich, Germany

Swi2/Snf2 ATPases are molecular machines that remodel substrate DNA:protein complexes. Most prominently, Swi2/Snf2 chromatin remodeller alter the position and composition of nucleosomes on DNA in a genome wide manner and ensure the correct structural organisation and dynamic properties of chromatin. Swi2/Snf2 remodeller form a very large and diverse family, ranging from smaller single subunit enzymes to large multisubunit chromatin remodeller such as the INO80 complex. INO80/ SWR1 family chromatin remodellers are complexes composed of >15 subunits and molecular masses exceeding 1 MDa. They have important roles in transcription, replication and DNA repair by exchanging the histone variants H2A and H2A.Z, as well as other emerging activities such as nucleosome positioning and sliding, but their mechanism, specificity and regulation is poorly understood. Using cryo-electron microscopy and X-ray crystallography, we have recently been able to derive structures of core remodeling modules of the INO80 complex at near atomic resolution. Our results reveal that large remodeling machines recognize nucleosomes and flanking DNA in a multivalent fashion through several modules and suggest a ratchet sliding mechanism underlying processive multistep remodelling. Nucleosome sliding is coupled to recognition of flanking DNA and requires functional communication between different modules. I will present our current understanding of the targeting, regulation and chemo-mechanical mechanism of the INO80 remodeller.

Structural studies of chromatin complexes

Song Tan Center for Eukaryotic Gene Regulation Penn State University University Park, PA. US

The packaging of DNA into chromatin allows our genetic material to fit into a nucleus, but it is also critical for both activation and repression of gene expression. This gene regulation is achieved through chromatin enzymes and factors which target the nucleosome repeating unit of chromatin.

My laboratory is interested in how chromatin enzymes and factors recognize their nucleosome substrates. Our crystallographic structures of the chromatin factor RCC1 and the Polycomb PRC1 ubiquitylation enzyme module bound to the nucleosome provide paradigms for how chromatin proteins can specifically interact with the both histone protein and DNA components of the nucleosome. I will discuss general principles that characterize how chromatin proteins bind to the nucleosome and I will present a new structure for a chromatin modification enzyme bound to the nucleosome.

Telomere-induced DNA damage as an anticancer strategy

Maria A. Blasco¹

¹Telomeres and Telomerase Group, Molecular Oncology Programme, Spanish National Cancer Centre (CNIO), Spain

Telomeres are considered as universal anti-cancer targets, as telomere maintenance is essential to sustain indefinite cancer growth. Mutations in telomerase, the enzyme that maintains telomeres, are among the most frequently found in cancer. In addition, mutations in shelterin components have been also reported in cancer. Although most efforts to target telomeres have focused in telomerase inhibition, recent studies suggest that direct targeting of the shelterin complex could represent a more effective strategy. In particular, we showed that genetic deletion of the shelterin component TRF1 impairs tumor growth in lung cancer and glioblastoma by direct induction of telomere damage independently of telomere length. Here, we screen for TRF1 inhibitory drugs and find that inhibition of several kinases of both the Ras and PI3K-AKT pathways, recapitulate the effects of Trf1 genetic deletion. We further show that such kinases phosphorylate TRF1 and that such modifications are essential for TRF1 stability. Finally, we use these new TRF1 regulatory pathways as the basis to discover novel drug combinations based on TRF1 inhibition, with the goal of effectively blocking potential resistance to individual drugs observed in glioblastoma.

DNA damage detection in nucleosomes involves DNA

Nicolas H. Thomä

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland University of Basel, Basel, Switzerland

Access to DNA packaged in nucleosomes is critical for gene regulation, DNA replication and repair. In humans, the UV-DDB complex detects ultraviolet light induced pyrimidine dimers throughout the genome, yet it remains unknown how these lesions are recognised in chromatin, where nucleosomes restrict DNA access. Here we report cryo-electron microscopy structures for UV-DDB bound to nucleosomes bearing a 6-4 pyrimidine-pyrimidone dimer (6-4PP), and a DNA damage mimic at a variety of positions. We find that UV-DDB binds UV damaged nucleosomes at lesions located in the solvent-facing minor groove without affecting the overall nucleosome architecture. For buried lesions facing the histone core, UV-DDB changes the predominant translational register of the nucleosome, and selectively binds the lesion in an accessible, exposed, position. These findings explain how UV-DDB detects occluded lesions in strongly positioned nucleosomes. We identify slide-assisted site-exposure (SAsSE) as a mechanism for high-affinity DNA-binding proteins to access otherwise occluded sites on nucleosomal DNA

Syota Matsumoto^{1,2*}, Simone Cavadini^{1,2*}, Richard D. Bunker^{1,2*}, Ralph S. Grand^{1,2}, Alessandro Potenza^{1,2,3}, Julius Rabl^{1,2}, Junpei Yamamoto⁴, Andreas D. Schenk^{1,2}, Dirk Schübeler^{1,2}, Shigenori Iwai⁴, Kaoru Sugasawa⁵, Hitoshi Kurumizaka^{6,7}, **Nicolas H. Thomä^{‡1,2}**

*Equal contribution

- ¹Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
- ²University of Basel, Basel, Switzerland
- ³Captor Therapeutics Ltd., Wroclaw, Poland

- ⁶Laboratory of Chromatin Structure and Function, Institute for Quantitative Biosciences,
- The University of Tokyo, Tokyo, Japan
- ⁷Graduate School of Advanced Science and Engineering, Waseda University, Shinjuku-ku, Tokyo, Japan

⁴Division of Chemistry, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka, Japan ⁵Biosignal Research Center, and Graduate School of Science, Kobe University, Kobe, Japan

Remodeling of RUVBL1-RUVBL2 ATPases by client proteins revealed by cryo-EM

Oscar Llorca

Spanish National Cancer Research Centre, Madrid, Spain

Phosphatidylinositol-3-kinase-like kinases (PIKKs) are a family of large protein kinases comprising several members (ATM, ATR, DNA-PKcs, TRAPP, SMG1 and mTOR), with essential roles in variety of cellular functions, such as DNA repair, DNA damage signalling, cell growth and nonsense-mediated mRNA decay. Assembly, cellular stability and activation of PI3-kinase-like kinases (PIKKs) require the action of HSP90 together with R2TP, a large multi-subunit HSP90 co-chaperone complex made of multiple subunits, including the RUVBL1 and RUVBL2 ATPases. Interestingly, other complexes such as RNA Pol II also require of this chaperone system for assembly, and the emerging view is that this is a specialised machinery for the assembly and stability of several large and complex macromolecular assemblies.

How HSP90 and the RUVBL1-RUVBL2-containing R2TP co-chaperone complex work in concert for the assembly and maturation of PIKKs and other complexes is poorly understood. As part of a joint collaboration with the group of Laurence H. Pearl (University of Sussex, UK), we are addressing these questions using cryo-electron microscopy (cryo-EM).

ATP binding and/or hydrolysis by the hexameric RUVBL1-RUVBL2 ATPases is essential for most of their documented functions in vivo, but structural studies show that access to the nucleotide-binding site is obstructed after hexamerization, and thus the mechanisms regulating nucleotide-exchange are unknown. We have used cryo-EM to reveal that PIH1D1, the client recruitment component of R2TP, regulates the access to the nucleotide-binding site in RUVBL1-RUVBL2. Our studies suggest a mechanism for the remodeling of these ATPases, which could be potentially shared by other RUVBL1-RUVBL2-containing complexes.

Conformational Activation Promotes CRISPR-Cas12a Catalysis and Resetting of the Endonuclease Activity

Stella S, Mesa P, Thomsen J, Paul B1, Alcón P, Jensen SB, Saligram B, Moses ME, Hatzakis NS, **Guillermo Montoya**

¹Structural Molecular Biology Group, Novo Nordisk Foundation Centre for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Conserved Research, Research, Sciences, University of Copenhagen, Sciences, Research, Sciences, Research, Sciences, Sc

Copenhagen, Denmark

²Department of Chemistry & Nanoscience Centre, University of Copenhagen, Copenhagen, Denmark

Cas12a, also known as Cpf1, is a type V-A CRISPR-Cas RNA-guided endonuclease that is used for genome editing based on its ability to generate specific dsDNA breaks. Here, we show cryo-EM structures of intermediates of the cleavage reaction, thus visualizing three protein regions that sense the crRNA-DNA hybrid assembly triggering the catalytic activation of Cas12a. Single-molecule FRET provides the thermodynamics and kinetics of the conformational activation leading to phosphodiester bond hydrolysis. These findings illustrate why Cas12a cuts its target DNA and unleashes unspecific cleavage activity, degrading ssDNA molecules after activation. In addition, we show that other crRNAs are able to displace the R-loop inside the protein after target DNA cleavage, terminating indiscriminate ssDNA degradation. We propose a model whereby the conformational activation of the enzyme results in indiscriminate ssDNA cleavage. The displacement of the R-loop by a new crRNA molecule will reset Cas12a specificity, targeting new DNAs.

Cell. 2018 Dec 13;175(7):1856-1871.e21.





Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response

Monday 20th

Session #2a DNA Replication and replication stress

Chairperson: Rafael Fernández-Leiro

Mechanisms for initiating DNA replication

James Berger

Johns Hopkins School of Medicine, Baltimore, US

RecA and AAA+ family ATPases play key roles in cellular events ranging from vesicle trafficking and proteolysis to chromatin remodeling and DNA replication/repair. A large subset of these enzymes form multimeric rings or spirals that respond to cycles of ATP binding and hydrolysis by undergoing changes in oligomeric and/or conformational state, and then transducing these physical rearrangements onto target macromolecules for controlling key biological processes. New structural and biochemical findings pertaining to how RecA/AAA+ ATPases and their accessory factors operate during the initiation of DNA replication will be discussed.

Operating principles and catalytic mechanism of DNA replisome

Wei Yang and Yag Gao

LMB, NIDDK, National Institutes of Health, NIH, Bethesda, US

DNA replication is essential for cell proliferation. The replisome that performs concerted leading and lagging DNA strand synthesis at a replication fork has never been visualized in atomic detail. Using bacteriophage T7 as a model system, we determined cryo-EM structures up to 3.2 Å of helicase translocating along DNA, and of helicase-polymerase-primase complexes engaging in synthesis of both DNA strands. Each domain of the spiral-shaped hexameric helicase translocates hand-over-hand sequentially along a ssDNA coil, akin to the way AAA+ ATPases unfold peptides. Two lagging-strand polymerases are attached to the primase ready for Okazaki-fragment synthesis in tandem. A β -hairpin from the leading-strand polymerase separates two parental DNA strands into a T-shaped fork, thus enabling the closely coupled helicase to advance perpendicular to the downstream DNA duplex. These structures reveal the molecular organization and operating principles of a replisome. Secondly, by combining in crystallo catalysis with time-resolved X-ray diffraction analysis, we have observed reaction intermediates of DNA synthesis at unprecedented atomic detail. Contrary to the conventional view that DNA synthesis occurs by a two-Mg²⁺-ion mechanism, we have discovered that two Mg²⁺ ions bound to the polymerase active site are insufficient for product formation. A third Mg²⁺ ion must be captured by the enzyme-substrate complex en route to product formation. This third metal ion is free of enzyme coordination and appears to drive phosphoryl-transfer by breaking the existing phosphodiester bond in dNTP. We find that cation trafficking in the DNA synthesis reaction is not an exception to the rule of the transition state theory, but it also drives hydrolysis by nucleases.

Structural features of High Fidelity DNA Replicartion

Meindert H. Lamers

Dep. of Cell and Chemical Biology Leiden University Medical Center, Leiden, The Netherlands https://ccb.lumc.nl/about-the-lamers-lab-68

Faithfull replication of the genome requires the combined action of a high-fidelity DNA polymerase, a 5'-3' exonuclease, and the post-replicative DNA mismatch repair. Our recent cryo-EM structures show how the polymerase-exonuclease work together to rapidly remove mismatched nucleotides during DNA replication. In contrast, post-replicative mismatch repair is a far more complicated process that requires a number of proteins that detect and remove mismatched nucleotides from millions of correctly matched base pairs. Here we present novel cryo-EM structures that reveal how the central mismatch repair enzyme MutS scans the DNA for mismatches, recognizes the mismatch, and subsequently recruits the second repair enzyme MutL. These structures reveal how MutS can scan millions of base pairs without triggering repair and how only mismatch binding will lead to a dramatic conformational change that elicits binding of MutL and the initiation of the repair cascade.

Molecular mechanisms of eukaryotic DNA replication and recombination

Luca Pellegrini

Cambridge University, Cambridge, UK

Our work aims to understand how accurate and efficient synthesis of chromosomal DNA is accomplished in human cells, under normal conditions and in situation of replicative stress. The genetic instructions required for the correct functioning of a human cell are encoded in its DNA. Each time a cell divides, it is crucial that two faithful and complete copies of its genome are transmitted to the daughter cells. This formidable biochemical task requires the intervention of complex molecular systems that copy the template DNA and repair lesions that stall DNA synthesis or degrade the encoded information. In addition to its biological importance, elucidating the molecular basis of DNA replication has great medical relevance. It is now recognised that disease states such as cancer, premature aging and neurodegenerative conditions are promoted by sporadic failure or inherited defects in the cellular mechanisms that protect our genome, which is at its most vulnerable during replication.

The biochemical study of DNA replication has now advanced to the point where the reaction of DNA synthesis has been reconstituted *in vitro* for the mono-cellular eukaryote budding yeast4. Much effort is correspondingly being put into the mechanistic analysis of the yeast replisome, the dynamic multi-protein assembly responsible for the synthesis of chromosomal DNA in S-phase. In our work, we propose to study the structural and biochemical basis for the more complex process of DNA replication taking place in human cells, and to investigate the recovery mechanisms acting when the human replisome stalls due to DNA damage or replication stress. In my lecture, I will discuss our latest experiments that seek to elucidate key molecular mechanisms of DNA replication and replication-coupled DNA repair using state-of-the art biochemical and biophysical approaches.

Eukaryotic DNA replication studied by cryo-electron microscopy

Patrik Eickhoff, Hazal Kose, Fabrizio Martino, Ferdos Abid Ali, Hasan Yardimci and Alessandro Costa

The Francis Crick Institute, London, UK

The eukaryotic replisome is a multicomponent complex containing a hexameric MCM helicase motor that unwinds the double helix and dedicated polymerases that copy DNA. We have solved the structure of the activated form of the helicase motor bound to DNA, imaged during ATPase-powered DNA translocation. We show that asymmetric DNA engagement around the MCM ring supports vertical movement through the helicase channel. Our data explain why not all six active sites are required for DNA unwinding. As we had previously solved the structure of a CMG complex bound to the leading strand polymerase, we can now explain how Pol epsilon modulates pausing of the replisome at a barrier. Taken together, our results provide new important insights into the mechanism of replisome progression in eukaryotic cells.

Cryo-EM structure of the human lagging strand DNA polymerase delta holoenzyme



¹Leicester Institute of Structural & Chemical Biology and Department of Molecular & Cell Biology,

²King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

³CIC bioGUNE, Parque Tecnológico de Bizkaia Edificio 800, Derio, Spain

DNA polymerases replicate the chromosomal DNA. In eukaryotes, DNA polymerase δ (pol δ) is responsible for replicating the lagging strand template and anchors to the proliferating cell nuclear antigen (PCNA) sliding clamp to form an active holoenzyme. The structural basis of pol δ activity is unclear. We have determined the 4.3 Å-resolution cryo-EM structure of the human heterotetrameric pol δ complex bound to primed DNA and PCNA in a replicating and pausing state. In both states, the polymerase is tethered to one of the three PCNA monomers only, with the catalytic core sitting on top of the clamp in an open configuration, and the regulatory subunits projecting laterally, allowing PCNA to thread the DNA exiting the catalytic cleft. Upon replication stalling, the DNA is released from the catalytic site via a motion of the polymerase thumb domain, which exposes the DNA primer-template junction, suggesting that the substrate may then be handed off to other DNA-editing enzymes pre-bound to PCNA. This work explains the molecular basis of pol δ processivity, and provides novel insight into the function of the human replisome.





University of Leicester, Lancaster Rd, Leicester, UK





Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response

Tuesday 21st

Session #2b DNA Replication and replication stress

Chairperson: Lori Passmore

The 3Rs (Recruitment, Role and Regulation) of PrimPol during DNA Replication in eukaryotic cells

Aidan Doherty

Genome Damage and Stability Centre, University of Sussex, Brighton, UK

DNA damage and secondary structures act as potent obstacles to the replication machinery. Persistent stalling leads to genomic instability and therefore numerous tolerance mechanisms exist to complete replication in the presence of such impediments. In addition to translesion synthesis (TLS) polymerases, eukaryotic cells also contain Primase-Polymerase (PrimPol), a replicative enzyme capable of repriming replication restart downstream of lesions / secondary structures, as well as directly bypassing DNA damage by TLS.

To elucidate the cellular requirements for PrimPol, we generated PrimPoldeleted cell lines (human & avian) and show that it plays key roles in maintaining efficient replication in both the nucleus and mitochondrion, even in the absence of exogenous damage. PrimPol-deficient cells are sensitive to genotoxins and exhibit delayed recovery after UV damage and increased mutation frequencies, micronuclei and sister chromatin exchanges. PrimPol is also required during mitochondrial replication, with null cells having increased mtDNA copy number but displaying a significant decrease in replication. Deletion of PrimPol in XPV cells, lacking functional polymerase Eta, causes an increase in damage sensitivity and pronounced fork stalling after UV treatment. We show that, unlike canonical TLS polymerases, PrimPol is important for allowing active replication to proceed, even in unperturbed cells, thus preventing the accumulation of excessive fork stalling and genetic mutations.

We demonstrate that PrimPol's primase activity is requisite to restore wild-type replication fork rates in PrimPol^{-/-} cells treated with genotoxins or encountering structures, establishing that repriming is a critical mechanism for replication restart in vertebrate cells. This capacity to reprime replication suggests that its deployment may be strictly regulated to prevent aberrant genome duplication. We show that PrimPol's is recruited to stalled forks by the RPA complex to facilitate replication restart. We identify PrimPol's RPA-binding motifs and demonstrate that this interaction is critical for its recruitment to stalled forks *in vivo* and stimulation of its primase activity *in vitro*.
A role for PrimPol at highly cytotoxic DNA lesions

Juan Méndez

DNA Replication Group, Molecular Oncology Programme, Spanish National Cancer Research Centre, Madrid, Spain

We are interested in the functions of PrimPol, the second primasepolymerase identified in mammalian cells after Pol α -primase. Previous work from our laboratory and others has revealed that PrimPol participates in the replicative tolerance of UV-induced DNA photoproducts by re-priming DNA synthesis downstream of the lesions, leaving short unreplicated gaps to be repaired post-replicatively. A related mechanism facilitates fork progression through natural obstacles such as G-quadruplex structures.

Using a synthetic lethality approach in CRISPR/Cas9-generated PrimPol KO human cells, we have found that loss of PrimPol sensitizes cells to the downregulation of the Fanconi Anemia pathway involved in the repair of inter-strand crosslinks (ICLs). ICLs are very cytotoxic lesions that hinder the progression of replication and transcription machineries, causing chromosome breakage, mutations and mitotic catastrophe when left unrepaired.

The canonical pathway of ICL repair involves the convergence of two replication forks at the crosslink, forming an X-shaped DNA structure that serves as the substrate for endonucleases, triggering a complex repair process that involves HR, NER and TLS polymerases. An alternative pathway involves a 'traverse' reaction in which the first fork that reaches the lesion is capable of bypassing it through the combined action of FANCM translocase, the Bloom syndrome helicase complex and a previously uncharacterized de novo priming event. Using a modification of the stretched DNA fiber technique that allows to monitor replication patterns specifically around ICL lesions, we have identified PrimPol as the key primase involved in the ICL traverse reaction, which is actually more common than the fork convergence mechanism. A functional complementation assay unequivocally indicates that PrimPol primase, and not its polymerase activity, is required for this early step in ICL recognition and repair. The *in vivo* implications of this new role of PrimPol have been tested in a viable PrimPol KO mouse strain and will be discussed

Structural studies of key components in DNA damage response

Xiaodong Zhang

Imperial College London, London, UK

Our research focuses on elucidating the structures and mechanisms of macromolecular complexes involved in DNA damage response, by combining biochemistry, X-ray crystallography and electron microscopy techniques. I will discuss our recent progress on a number of key components involved in recruitment and signalling during this process. One crucial player is the Replication Protein A (RPA) which binds to single stranded (ss) DNA and interacts with a myriad of proteins, thus act to protect ssDNA from nucleolytic degradation and serve as a platform for recruiting and assembly other signalling and repair proteins. I will discuss our structural and mechanistic studies on RPA assembly on ssDNA.



CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

Tuesday 21st

Session #3a DNA transcription

Chairperson: Lori Passmore

cnio stop cancer

TFIIH in transcription and DNA repair

Patrick Cramer

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Our laboratory combines structural biology with functional genomics and computational biology to study the mechanisms of gene transcription and its regulation in eukaryotic cells. Recent work includes structural studies of chromatin remodeling by Chd1 (Farnung Nature 2017) and Pol II initiation (Plaschka Nature 2015, 2016; Nozawa Nature 2017; Schilbach Nature 2017). We also developed transient transcriptome sequencing (TTseq), which can map changes in RA synthesis and enhancer landscapes at high temporal resolution (Schwalb Science 2016; Demel Mol. Syst. Biol. 2017). We recently also resolved structures of paused and activated Pol II elongation complexes (Vos, Farnung et al., unpublished) together with in vivo evidence for polymerase pausing during elongation controlling initiation (Gressel, Schwalb et al. Leonhardt, Eick, and Cramer, eLife 2017). In my talk I will concentrate on unpublished work from the laboratory that is relevant for understanding DNA nucleotide excision repair (NER). In particular, we have analyzed the large conformational changes that occur in the 10-subunit factor TFIIH when it switches from a function in transcription to a role DNA repair, and have investigated the underlying regulatory mechanisms.

Unveiling RNA Polymerase III (extra)transcriptional complex with EM

Alessandro Vannini

Institute of Cancer Research, ICR, London, UK

RNA polymerase (Pol) III transcribes essential RNAs, including the entire pool of tRNAs, the 5S ribosomal RNA and the spliceosomalU6 snRNA, with the help of transcription factors TFIIIB and TFIIIC. Recent findings point to a broader role of the Pol III apparatus in several processes, such as retrotransposon's integration, nucleosome positioning, global and subnuclear organization and control of pervasive Pol II transcription. These "extra-transcriptional" functions depends on coordinated interactions between the Pol III apparatus and other macromolecular machineries, in the context of specific chromatin structures. Exploiting recent advances in electron microscopy and integrating the structural analysis with functional data in vitro and in living cells, we are elucidating the molecular mechanisms underlying Pol III transcription and its extra-transcriptional role in the spatial and functional organisation of the human genome.



Structural basis of RNA polymerase I stalling at UV light-induced DNA damage

Marta Sanz-Murillo¹, Jun Xu², Georgiy A. Belogurov³, Olga Calvo⁴, David Gil-Carton⁵, María Moreno-Morcillo⁶, Dong Wang², **Carlos Fernández-Tornero**¹

¹CIB-CSIC, Madrid, Spain ²Skaggs School of Pharmacy & Pharmaceutical Sciences, UCSD, USA ³University of Turku, Finland ⁴IBFG, CSIC-USAL, Salamanca, Spain ⁵CIC-bioGUNE, Derio, Spain ⁶CBMSO-CSIC, Madrid, Spain

DNA lesions threaten cell life and must be repaired to maintain genome integrity. During transcription, RNA polymerases actively scan DNA to find bulky lesions and trigger their repair. In growing eukaryotic cells, about 60% of the total transcriptional activity involves the synthesis of ribosomal RNA (rRNA) by RNA polymerase I (Pol I), a 14-subunit macromolecular machine with unique regulatory features [1]. Accordingly, Pol I monitors rDNA integrity and influences cell survival, but little is known about how this macromolecular machine processes UV lightinduced lesions. We determined the cryo-EM structure of Pol I stalled by a UVinduced lesion, cyclobutane pyrimidine dimer (CPD), at 3.6 Å resolution to unveil how the enzyme manages this important DNA damage [2]. The structure shows that the lesion induces an early translocation intermediate with a peculiar arrangement in the vicinity of the active site. In particular, the bridge helix residue Arg1015 contributes to CPD-induced Pol I stalling, as confirmed by mutational analysis. These results, together with biochemical data, reveal how Pol I stalls at CPD lesions, which is distinct from Pol II arrest by this DNA damage. Our findings open the avenue to unravel the molecular mechanisms underlying cell endurance to lesions on ribosomal DNA.

¹Torreira E, Louro JA, Pazos I, González-Polo N, Gil-Carton D, Duran AG, Tosi S, Gallego O, Calvo O, Fernández-Tornero C (2017) The dynamic assembly of distinct RNA polymerase I complexes modulates rDNA transcription. eLife 6:e20832

²Sanz-Murillo M, Xu J, Belogurov GA, Calvo O, Gil-Carton D, Moreno-Morcillo M, Wang D, Fernández-Tornero C (2018) Structural basis of RNA polymerase I stalling at UV light-induced DNA damage. Proc. Natl. Acad. Sci. USA 115(36):8972-8977



CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

Tuesday 21st

Session #3b DNA transcription

Chairperson: Wei Yang

cnio stop cancer

Molecular visualization of the transcription initiation

Eva Nogales

Molecular and Cell Biology, UC Berkeley Howard Hughes Medical Institute Lawrence Berkeley National Laboratory Berkeley, US

We are using cryo-EM in the study of complex gene expression machinery for which only small amounts of sample are available, and that suffer from both compositional and conformational mixtures. The latter shortcomings reflect the dynamic nature of the biological processes these protein complexes are involved with, and overcoming them and describing the functionally relevant heterogeneity of biological systems is one of the novel values of the cryo-EM methodology.

A major effort in our lab for many years has been to describe the structure and dynamics of human TFIID, a complex of over 1 MDa that binds to DNA and recruits the rest of the transcription initiation machinery. TFIID is composed of TBP (TATA-binding protein) and 13 TAFs (TBPassociated factors). Our studies provide the first, full subunit organization within this large complex. They also describe the structural heterogeneity of human TFIID and how it relates to the critical process of core promoter recognition and binding, most significantly, how dynamics of one of TFIID structural lobes is used for the controlled loading of TBP onto the upstream DNA core promoter region. We have also obtained the structure of human TFIIH, a complex essential both in transcription initiation and in base excision DNA repair. Our structure reveals how the helicase XPD, essential in NER, is inhibited within the apo TFIIH by a complex set of interaction with other subunits that need to be relieved for TFIIH to function in the repair process.

The molecular mechanism of TFIIH recruitment to DNA damage-stalled RNA polymerase II during transcription-coupled DNA repair

Martijn S. Luijsterburg

Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

The response to DNA damage-stalled RNA polymerase II (RNAPIIo) involves the assembly of the transcription-coupled repair (TCR) complex on actively transcribed strands. The function of the TCR proteins CSB, CSA and UVSSA and the manner in which the core DNA repair complex, including transcription factor IIH (TFIIH), is recruited is largely unknown. Here, we define the assembly mechanism of the TCR complex in human isogenic knockout cells. We show that TCR is initiated by RNAPIIo-bound CSB, which recruits CSA through a newly identified CSA-interaction domain (CID). Once recruited, CSA facilitates the association of UVSSA with stalled RNAPIIo. Importantly, we find that UVSSA is the key factor that regulates the assembly of the TFIIH complex in a manner that is stabilized by CSB and CSA. Together these findings reveal a sequential and highly cooperative assembly mechanism of TCR proteins and reveal the mechanism of TFIIH recruitment to DNA damage-stalled RNAPIIo to initiate repair.



Molecular basis of tRNA recognition by the Elongator complex

Maria I. Dauden^{1,7}, Marcin Jaciuk², Felix Weis¹, Lin TY², Carolin Kleindienst³, Nour El Hana Abbassi^{2,4}, Heena Khatter¹, Rościsław Krutyhołowa^{2,5}, Karin D. Breunig³, Jan Kosinski^{1,6*}, Christoph W. Müller^{1*} and Sebastian Glatt^{2*}

¹European Molecular Biology Laboratory, Heidelberg, Germany
²Max Planck Research Group at the Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland
³Institute of Biology, Martin Luther University Halle-Wittenberg, Halle, Germany
⁴Postgraduate School of Molecular Medicine, Warsaw, Poland
⁵Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Poland
⁶Centre for Structural Systems Biology, DESY and European Molecular Biology Laboratory Hamburg, Germany
⁷Current adress: CNIO, Madrid, Spain

The highly conserved Elongator complex modifies tRNAs in their wobble base position, thereby regulating protein synthesis and ensuring proteome stability. The precise mechanisms of tRNA recognition and its modification reaction remain elusive. Here, we show cryoelectron microscopy structures of the catalytic subcomplex of Elongator and its tRNA-bound state at 3.3 and 4.4 Å resolution. The structures resolve details of the catalytic site, including the substrate tRNA, the iron-sulfur cluster and a SAM molecule, which are all validated by mutational analyses *in vitro* and *in vivo*. tRNA binding induces conformational rearrangements, which precisely position the targeted anticodon base in the active site. Our results provide the molecular basis for substrate recognition of Elongator, essential to understand its cellular function and role in neurodegenerative diseases and cancer.





CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response

Tuesday 21st

Session #4a DNA damage and repair

Chairperson: Wei Yang

cnio stop cancer

Phosphorylation-dependent assembly of DNA damage response complexes

Laurence H. Pearl

Genome Damage and Stability Centre-University of Sussex, Brighton, UK

Coordination of the cellular response to DNA damage is organised around a set of large multi-domain 'scaffold' proteins, including BRCA1, MDC1, 53BP1 and TOPBP1, which recognise post-translational modifications such as phosphorylation, methylation and ubiquitylation on other proteins, and are themselves carriers of such regulatory signals. My laboratory is studying how these post-translational modifications, especially phosphorylation, provide the signals for assembly of the multi-protein complexes that mediate DNA damage signalling and repair. I will discuss published work on assembly of the DNA damage checkpoint complex, and present new data on a phosphorylation-dependent collaboration between 53BP1 and TOPBP1 that has a major regulatory role in preventing replication in the presence of DNA damage.

Mechanistic insights into DNA repair using cryo-EM

Lori Passmore

MRC Laboratory of Molecular Biology, Cambridge, UK

The Fanconi Anemia (FA) pathway repairs DNA damage caused by endogenous and chemotherapy-induced DNA crosslinks. Genetic inactivation of this pathway impairs development, prevents blood production and promotes cancer. The key molecular step in the FA pathway is the monoubiquitination of a heterodimer of the FANCI-FANCD2 substrate proteins by the FA core complex - a megadalton multiprotein E3 ubiquitin ligase. Monoubiquitinated FANCI-FANCD2 then initiates removal of the DNA crosslink. Lack of molecular insight into the FA core complex limits a detailed explanation of how this DNA repair pathway functions. The complex contains eight different subunits, most with unknown structure and no substantial homology to proteins of known structure. Moreover, the functions of many of the subunits are unclear. We aim to understand the mechanism of the FA core complex by biochemically reconstituting the specific monoubiquitination reaction, and by determining structures of the FA proteins. We now report purification and structural characterization of an intact FA core complex. Together our data allow us to propose a model for the architecture of the entire FA core complex, providing new insight into the structure and function of its subunits.



A conformationally versatile complex between Ctf18-RFC and DNA Pol ϵ is required for leading strand replication stress signaling

Alicja Winczura, Katy Stokes, Boyuan Song, Giacomo de Piccoli and **Daniel B. Grabarczyk**

¹University of Warwick, Warwick Medical School, Coventry, UK ²Department for Structural Biology, Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Germany

Ctf18-RFC is an alternative PCNA loader with a specialized role at the replication fork that is critically important for replication stress signaling and cohesion establishment. The clearest difference between Ctf18-RFC and the standard PCNA loader, Rfc1-RFC, is its interaction with the leading strand DNA Polymerase ε via its Ctf18-1-8 module. We have characterized the complex formed between Ctf18-1-8 and the entire catalytic domain of Pol ɛ using an integrative structural biology approach in combination with yeast genetics. We first show that the interaction does not interfere with the polymerase activity of Pol ε , and that the interaction is very tight in both the absence and presence of a DNA substrate. This means that Ctf18-RFC is likely to form a stable part of the leading strand machinery during normal replication and fork stalling. We present the 4.2 Å crvo-EM structure of the complex, which reveals a much larger interface than previously seen in a minimal complex. Surprisingly, Ctf18-1-8 binds across multiple Pol ε subdomains that move during DNA binding and catalysis. By comparing different classes from cryo-EM analysis and a new crystal form of the minimal complex, we show that large slippery interaction patches on Pol ε enable extremely versatile binding of Ctf18-1-8 to different Pol ε conformations.

We then use structure-guided mutagenesis to specifically disrupt recruitment of Ctf18-RFC to Pol ε in vivo. We show that positioning of Ctf18-RFC at the leading strand is critical for the replication stress response but is compensated by the lagging strand stress signaler, Rad24-RFC, defining parallel leading and lagging strand signaling pathways. The mutations also impact epigenetic maintenance, but surprisingly cohesion establishment is unaffected. This separation of function enables us to understand the roles that Ctf18-RFC and other replication-associated cohesion establishment factors, such as Chl1, Mrc1 and Ctf4, play during normal DNA replication.



CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response

Wednesday 22nd

Session #4b DNA damage and repair

Chairperson: Oscar Llorca

cnio stop cancer

Charting the response to DNA damage using functional genomics

Daniel Durocher

The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada durocher [at] lunenfeld [dot] ca

The orchestration of DNA repair is of fundamental importance to the maintenance of genomic integrity and tumor suppression. DNA damage must be detected in the context of the varied chromatin landscape, its presence must be communicated throughout the cell to alter many ongoing processes, and the machinery that will mend the lesion must be recruited to the damage site. In my presentation, I will discuss our recent efforts in mapping genome maintenance pathways using genome-scale CRISPR/Cas9 screens in human cells. I will highlight how these screens can be used to identify new genome stability factors, characterize drug responses and provide new insights into the genetic architecture of the genome stability network by identifying potentially actionable synthetic lethal genetic interactions. I will argue that somatic genetic screens in human cells are powerful tools to study the DNA damage response and its integration within other cellular pathways.

Structural mechanisms of regulation of PIKK family members

Roger Williams

MRC Laboratory of Molecular Biology, Cambridge UK

ATM and mTOR are both members of the phosphatidylinositol 3-kinase-related protein kinase (PIKK) family of enzymes. Cryo-EM is elucidating aspects of the structures and regulation of members of this family. Despite having closely related kinase domains, the structures suggest a wide range of regulatory mechanisms controlling their activities. ATM has an important role in DNA double-strand break repair. Our cryo-EM structure suggests that human ATM is in a dynamic equilibrium between closed and open dimers, with the PIKK regulatory domain (PRD) having a conformation that is dictated by this dynamic equilibrium so that it acts inhibits the enzyme in the closed conformation. The dimer interface is formed by the FAT domains of paired protomers, and pivoting around this interface controls the conformation of the active site. The mTOR-containing complex mTORC1 is regulated by two types of G-proteins, RHEB that allosterically activates the enzyme complex and the Rags that control its activity by localisation. Allosteric regulation appears to be a feature of both ATM and mTOR, but details of the mechanism differ widely. Nevertheless, for both of these PIKKs conformational changes of the FAT domain feature in the regulatory mechanism. Control of mTORC1 by localization to membranes depends on the unique heterodimeric Rag GTPases. Localization of ATM to sites of DNA damage is also a key part of the mechanism of activation. However the structures and dynamics of the scaffolds that achieve localization in ATM and mTOR are unrelated

- Domagoj Baretić¹, Madhanagopal Anandapadamanaban¹, Glenn R. Masson¹, Olga Perisic¹, Alex Berndt¹, Chris M. Johnson¹, Hannah K Pollard², Balaji Santhanam¹, David I Fisher², Caroline Truman², Jonathan Kaufman¹, Tiomas Kouba¹, Alan Fersht¹, Kacper B. Rogala³, Christopher Phillips², David M. Sabatini³, **Roger Williams¹**
- ¹MRC Laboratory of Molecular Biology, Cambridge UK

²Discovery Sciences, Innovative Medicines and Early Development Biotech Unit, AstraZeneca, Cambridge UK ³Whitehead Institute for Biomedical Research, Cambridge



Structural basis of allosteric regulation of Tel1/ATM kinase

Jiyu Xin^{1,†}, Zhu Xu^{1,†}, Xuejuan Wang^{1,2,†,*}, Yanhua Tian³, Zhihui Zhang1 and **Gang Cai^{1,2,*}**

¹First Affiliated Hospital of USTC, School of Life Sciences, University of Science and Technology of China, Hefei, China

²Hefei National Laboratory for Physical Sciences at Microscale and

CAS Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, Hefei, China ³College of Life Science and Technology, Huazhong University of Science and Technology,

Wuhan, Hubei, China

The ATM/Tel1 protein is an apical kinase that orchestrates the multifaceted DNAdamage response, mutations of which are associated with ataxia telangiectasia syndrome. Here we report cryo-EM structures of symmetric dimer (4.1 Å) and asymmetric dimer (4.3 Å) of S. cerevisiae Tel1. In the symmetric state, the side chains in Tel1 C-terminal (residues 1,129-2,787) are discernible and an atomic model is built. The substrate binding groove is completely embedded in the symmetric dimer by the intramolecular PRD and intermolecular LID domain, where point mutations sensitize the S. cerevisiae cells to DNA damage agents and hinder Tel1 activation due to reducing the binding affinity for its activator-Xrs2/Nbs1. In the asymmetric state, one monomer becomes more compact in two ways: the kinase N-lobe moves down and the Spiral of α -solenoid moves upwards, which resemble the conformational changes observed in active mTOR. The accessibility of the activation loop correlates with the synergistic conformational disorders in the TRD1-TRD2 linker, FATC and PRD domains, where critical posttranslational modifications and activating mutations are coincidently condensed. This study reveals a tunable allosteric network in ATM/Tel1, which are important for substrate recognition, recruitment and efficient phosphorylation.

Binding and unwinding activities of human HELB unveiled by biochemical and biophysical methods



Silvia Hormeño¹, Oliver Wilkinson², Clara Aicart-Ramos, Mark S. Dillingham² and $Fernando\ Moreno-Herrero^1$

¹Department of Macromolecular Structures, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain ²School of Biochemistry, University of Bristol, Bristol, UK

Homologous recombination (HR) is a DNA repair mechanism that is initiated by the resection of a DNA end to generate a 3'-terminated ssDNA tail. It necessarily requires the presence of a sister chromatid produced by DNA replication. Thus, HR is highly regulated to be suppressed during G1 and activated as cells enter S phase. DNA helicase B (HELB) is 5'-3' helicase that inhibits DNA end resection in G1 phase by blocking the EXO1 and BLM-DNA2 DNA-end resection pathways. However, despite its important role in the regulation of DSB repair, the molecular mechanism behind DNA unwinding and DNA-end resection inhibition by HELB is mostly unknown. Here, we present a biochemical and biophysical study of the unwinding activity of HELB. We first characterized human HELB biochemical activities. We found that HELB tightly binds to ssDNA and it has ssDNAdependent ATPase activity. Gel-based assays also showed that HELB still binds to DNA coated with human RPA with similar affinity to naked DNA, whereas binding is completely inhibited if DNA is covered with bacterial SSB. We then developed an assay based on magnetic tweezers to study the unwinding activity of HELB. Kilobasepair-long DNA molecules were produced containing an internal 5'- terminated poly-T flap, which served as a substrate for HELB. HELB unwound the duplex with 5'-3' polarity at rates of \sim 30 bp s-1. Duplex unwinding resulted in increment of DNA extension at high forces, where ssDNA cannot form secondary structures. Eventually, we also observed reduction of extension consistent with the formation of a loop ahead of the enzyme. At low forces the extension of the tether always decreased due to HELB unwinding activity and the formation of ssDNA secondary structures. Data at high and low stretching forces are compatible with a model where HELB translocates with 5'-3' polarity but is able to switch strands therefore producing unwinding and rewinding of the duplex DNA.



CryoEM Structure of the XPF-ERCC1 Endonuclease Reveals an Auto-Inhibited Conformation

Morgan Jones, Fabienne Beuron, Andrea Nans, Aaron Borg, Maureen Bowles, Edward Morris, Mark Linch, Neil Q McDonald

The Francis Crick Institute, The Institute of Cancer Research, The UCL Cancer Institute, London, UK

During the DNA damage response, the activity of DNA repair nucleases must be tightly regulated to prevent promiscuous cleavage and genomic instability. The XPFERCC1 (XE) endonuclease is a structure-specific 5'-3' nuclease with key roles in both nucleotide excision repair (NER) and inter-strand cross-link repair (ICLR). It is not fully understood how XE is recruited into discrete repair pathways or how XE activity is regulated. Here we describe the first full-length structure of human XE, solved using cryo-EM, revealing an auto-inhibited conformation. Patient derived Fanconi-anaemia mutations, deficient in ICLR but proficient in NER, cluster in the XPF helical domain implicating this site as critical for ICLR recruitment and activation. Our results demonstrate how nuclease activity is regulated in XPF/MUS81 family endonucleases and rationalises the role of the XPF helicase-like domain which both obscures the ERCC1 DNA binding hairpins and regulates DNA access to the active site. High ERCC1 expression in a number of cancers has been linked to a poor response to platinum-based chemotherapy. We hope that this structure will provide a starting point for the rational design of XE inhibitors to overcome chemoresistance.





CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20-22 May 2019

Structural and molecular biology of the DNA damage response

Organisers & Speakers' Biographies

cnio stop cancer



James Berger Johns Hopkins School of Medicine, Baltimore, US

James Berger is Professor of Biophysics and Director of the Institute for Basic Biomedical Sciences at the Johns Hopkins School of Medicine. Prior to Berger's arrival at Hopkins in 2013, he served on the faculty at the University of California, Berkeley. Berger earned his Ph.D. from Harvard University and was a Whitehead Fellow at MIT. Berger's research focuses on understanding how the structure and mechanism of molecular machineries help control DNA replication, gene expression, and chromosome topology. He is a member of the American Academy of Arts and Sciences, the National Academy of Medicine, and the National Academy of Sciences.



Maria A. Blasco

Director of the *Centro Nacional de Investigaciones Oncológicas* Head of the Telomeres and Telomerase Group Spanish National Cancer Research Centre (CNIO) Madrid, Spain

Maria A. Blasco obtained her PhD in 1993 at the *Centro de Biología Molecular "Severo Ochoa"* under the supervision of M. Salas. That same year, Blasco joined the *Cold Spring Harbor Laboratory* in New York (USA) as a Postdoctoral Fellow under the leadership of C. W. Greider. As a postdoc she isolated one of the telomerase essential genes and generated the first telomerase deficient mouse model, which served to demonstrate the importance of telomerase in telomere maintenance, chromosomal instability and disease. In 1997, she returned to Spain to start her own research Group at the *Centro Nacional de Biotecnología* in Madrid. She joined the *Spanish National Cancer Research Center (CNIO)* in 2003 as Director of the Molecular Oncology Programme and Leader of the Telomeres and Telomerase Group. In 2005, she was also appointed Vice-Director of Basic Research at CNIO. Since June 2011, she is the CNIO Director.

For more than 20 years, Blasco's work has focused in demonstrating the importance of telomeres and telomerase in cancer, as well as in age-related diseases. Blasco has published more than 250 papers in international journals and has an h-index of 81. Her achievements have been recognized by the following international and national awards: Josef Steiner Cancer Research Award, Swiss Bridge Award for Research in Cancer, Körber European Science Award, the EMBO Gold Medal, the "Rey Jaime I" Award in Basic Research, the Fundación Lilly Preclinical Research Award, and the "Santiago Ramón y Cajal" National Award in Biology. Blasco holds two Doctorate Honoris Causa from the *Universidad Carlos III* of Madrid and from *Universidad de Alicante*.



Alessandro Costa The Francis Crick Institute, London, UK

Alessandro Costa obtained a PhD in Structural Biology working in Silvia Onesti's laboratory at Imperial College London, studying archaeal ATPase proteins by EM. After a spell in Oxford working with Steve D. Bell on the biochemistry archaeal genome replication, Alessandro moved to Berkeley, to work with James Berger on the mechanisms of helicase activation. In 2012 Alessandro started his group at the London Research Institute Clare Hall laboratories (now part of the Crick Institute). Alessandro's group employ cryo-electron microscopy to study eukaryotic DNA replication.



Patrick Cramer Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Patrick Cramer is Director at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. His laboratory studies the mechanisms of gene transcription and its regulation in eukaryotic cells. They combine structural biology with functional genomics and computational modeling. The Cramer laboratory has analyzed the structure and function of RNA polymerase complexes with many different factors and arrived at models for transcription initiation and elongation. Cramer's long-term aim is to understand the expression and regulation of the genome on a molecular level. For his research work, Cramer has been awarded several prizes, such as the Feldberg Foundation Prize (2011), the Ernst-Jung Award for Medicine (2009) and the Gottfried-Wilhelm-Leibniz Award (2006). In 2012 he received the Federal Cross of Merit from the Federal Republic of Germany. Cramer is an elected member of the European Molecular Biology Organization (EMBO) and the German Academy of Sciences (Leopoldina).

Homepage: http://www.mpibpc.mpg.de/cramer

LinkedIn: https://de.linkedin.com/in/patrick-cramer-813073131

Twitter: https://twitter.com/CramerLab



Aidan Doherty

Professor of Biochemistry Genome Damage & Stability Centre, University of Sussex, Brighton, UK

After obtained his PhD from the University of Southampton, Aidan undertook postdoctoral fellowships working on DNA metabolic enzymes. In 2008, he set up his own group at the University of Cambridge. In 2003, he moved to the Genome Damage and Stability Centre in Brighton. His research programme is directed at identifying and characterizing novel protein complexes, which mediate the repair and bypass of specific lesions / structures that arrest genome replication. His group adopts multi-disciplinary approaches to discover novel DNA repair / replication pathways and determine how these operate, at both the cellular and molecular levels, to maintain genome stability.


Daniel Durocher The Lunenfeld-Tanenbaum Research Institute, Toronto, Canada

Daniel Durocher is a Senior Investigator and a Biomedical Program Director at the Lunenfeld-Tanenbaum Research Institute. He is also a Professor in the Department of Molecular Genetics at the University of Toronto.

Dr. Durocher's overarching interest lies in understanding how cells maintain genome integrity, with an emphasis on the detection, signaling and repair of DNA double-strand breaks. He is also the founder of Repare Therapeutics, an early-state drug development company aiming to develop new drugs based on the concept of synthetic lethality.

Among the prizes, awards and honors received by Dr. Durocher in recent years, particularly notable is the 2016 Paul Marks Prize for cancer research awarded by Memorial Sloan Kettering.



Rafael Fernández Leiro

Spanish National Cancer Research Centre, Madrid, Spain

Rafael Fernandez Leiro is a Junior Group Leader at the Spanish National Cancer Research Centre (CNIO).

He obtained his PhD in 2011 from the University of A Coruña in Spain where he worked in protein engineering using directed evolution techniques and structure-led rational design. During his PhD he visited the Rocasolano Institute in Madrid where he was trained in X-ray crystallography. After obtaining the PhD he joined the Lamers lab at the LMB in Cambridge to work on DNA repair and replication. During the 6 years that he spent at the LMB he worked with Meindert Lamers and Sjors Scheres to study multiple DNA replication and repair complexes and understand how these systems are regulated using cryo-EM and biochemistry.

He has recently joined CNIO to lead the "Genome Integrity & Structural Biology" group that focuses on the study of the function and biological implications of macromolecular complexes responsible for the maintenance of the integrity of the genome and the role they play in disease.



Karl-Peter Hopfner

Gene Center and Department of Biochemistry Ludwig-Maximilians-University Munich, Munich, Germany

- PhD in Biochemistry, Max-Planck-Institute of Biochemistry, Martinsried and TU Munich
- Postdoctoral research, Max-Planck-Institute of Biochemistry, Martinsried, Germany
- Postdoctoral research, The Scripps Research Institute, La Jolla
- Professor of Biochemistry, Gene Center,
- Ludwig-Maximilians-Universität, Munich

Research: Westudyhow cells shape and protect their genomic infor-mation. Using cryo-EM, X-ray crystallography and functional approaches, we study the architectures and mechanisms of macromolecular complexes in genome biology with a focus on chromatin remodelers, DNA double-strand break repair enzymes and innate immune nucleic acid sensors.



Meindert H. Lamers Dep. of Cell and Chemical Biology Leiden University Medical Center

Leiden. The Netherlands

Meindert H. Lamers obtained his master's degree from the University of Amsterdam, The Netherlands. He then started his PhD under the guidance of Titia K. Sixma at the Netherlands Cancer Institute, Amsterdam. Following his PhD he moved to the USA as a post-doctoral fellow in the laboratory of John Kuriyan, at the University of California, Berkeley. In 2009 he started his own group at the Laboratory of Molecular Biology, Cambridge, United Kingdom. In 2017 he moved back to the Netherlands, where the continuous his research at the Leiden University Medical Center.



Oscar Llorca

Director of the Structural Biology Programme, Macromolecular Complexes in DNA Damage Response Group Leader, Spanish National Cancer Research Centre, Madrid, Spain

Oscar Llorca obtained his Ph.D at the CNB-CSIC (Madrid) under the supervision of JL Carrascosa and JM Valpuesta, working on the structural characterization of chaperones using electron microscopy. He joined the Chester Beatty Laboratories (Institute of Cancer Research, London) in 2000 as a postdoct to characterize DNA repair complexes under the supervision of Keith R. Willison and Alan Ashworth. In June 2002, he became group Leader at the CIB-CSIC in Madrid. Since July 2017, Llorca leads the CNIO Structural Biology Programme. Llorca applies cryo-EM methodologies to the study of macromolecular complexes and molecular mechanisms in the DNA damage response.



Juan Méndez

DNA Replication Group Leader, Molecular Ongology Programme. Spanish National Cancer Research Centre, Madrid, Spain

J. Méndez (PhD, 1995) was a graduate student under the supervision of Margarita Salas and Luis Blanco (Centro de Biología Molecular "Severo Ochoa", Madrid) working on bacteriophage DNA replication. As a postdoctoral fellow in the group of Bruce Stillman (Cold Spring Harbor Laboratory, New York), he studied the functions of ORC, CDC6 and MCM2-7 proteins in human cells. He joined the CNIO Faculty in 2004. Research highlights from his group include the identification of dormant replication origins that are activated in response to stress, the role of cohesin at DNA replication factories, and the functional characterization of human DNA primase/polymerase PrimPol.



Eva Nogales

Professor, Molecular and Cell Biology Howard Hughes Medical Institute Investigator Senior Faculty Scientist, LBNL Berkeley, US

Born in Madrid, Eva Nogales obtained her Bachelors Degree in Physics from the *Universidad Autónoma de Madrid*. She carried out her graduate studies at the Synchrotron Radiation Source in Daresbury Laboratory (U.K) where she used SAXS and cryo-EM in the study of drug-induced tubulin self-assembly. Nogales moved to Berkeley to carry out postdoctoral work in the lab of Ken Downing at the Lawrence Berkeley National Lab. There she used electron crystallography to solved the atomic structure of tubulin. Nogales then joined the faculty at UC Berkeley, where she is presently a Professor in the Molecular and Cell Biology Department at the University of California Berkeley and where she serves as the Head for the Biochemistry, Biophysics and Structural Biology Division. Nogales is also a Senior Faculty Scientist within the Molecular Biophysics and Integrated Bio-Imaging Division at the Lawrence Berkeley National Lab, and an Investigator of the Howard Hughes Medical Institute.

Eva Nogales studies the mechanism of function and regulation of the microtubule cytoskeleton and the molecular machinery involved in eukaryotic gene regulation. In her studies she uses cryo-EM to describe structure, dynamics and interactions of these critical eukaryotic systems.

Eva Nogales is the recipient of a number of awards, among them the Dorothy Hodgkin Award from the Protein Society, the Mildred Cohn Award from the American Society for Biochemistry and Molecular Biology, the Porter Lecture Award from the American Society for Cell Biology, and the Grimwade Medal by the University of Melbourne. She is a member of the National Academy of Sciences and the American Academy of Arts and Sciences.



Lori A Passmore, PhD

Programme Leader MRC Laboratory of Molecular Biology, Cambridge UK passmore[at]mrc-Imb.cam.ac.uk

Lori Passmore studied Biochemistry at the University of British Columbia in Vancouver and received her PhD from The Institute of Cancer Research in London, working with David Barford. She was then a post-doc at the MRC Laboratory of Molecular Biology in Cambridge with Venki Ramakrishnan and Richard Henderson. Both her PhD and post-doctoral work focused on the structure and function of large macromolecular assemblies using cryo-EM. Since 2009, she has been a group leader at MRC LMB. Her work focuses on understanding the mechanisms of macromolecular protein complexes involved in regulating gene expression. She uses an integrated approach combining structural, biochemical and functional studies, aiming to reconstitute multi-protein complexes and their activities, and determine their high-resolution structures to understand their mechanisms. Lori's lab also developed new supports for cryo-EM that reduce image blurring. She was elected as a member of EMBO in 2018.



Laurence H. Pearl Genome Damage and Stability Centre-University of Sussex, Brighton, UK

Professor Laurence Pearl is Professor of Structural Biology in the Genome Damage and Stability Centre at the University of Sussex, and Head of the Division of Structural Biology at the Institute of Cancer Research in London.

His research focuses on understanding the structural basis for recognition, repair and signalling of DNA damage and the function of molecular chaperones, and translating this basic research for the discovery of new drugs.

He is a member of EMBO and a Fellow of the Royal Society, and was awarded the 2018 Novartis Medal and Prize by The Biochemical Society.



Luca Pellegrini Cambridge University, Cambridge, UK

I am currently a Professor of Structural Biology at the University of Cambridge, UK. I received my PhD in Natural Sciences at the ETH, Zürich, Switzerland in 1996, and joined the Department of Biochemistry of the University of Cambridge for post-doctoral research (1997-2003). In 2003, I received a Wellcome Trust Senior Research Fellowship award to study the structural biology of eukaryotic double-strand DNA break repair. Since then, the aim of our research has been to define atomic structure and mechanism of action of protein complexes that protect and replicate our DNA.



Titia Sixma Netherlands Cancer Institute, NKI, Amsterdam, Netherlands

Titia Sixma uses a combination of structural biology, biophysics and biochemistry to quantitatively analyze molecular mechanisms in cells. Her lab is interested in DNA regulation by ubiquitin and DNA mismatch repair. She received her PhD in Groningen with Wim Hol and did her post-doc with Paul Sigler at Yale. She is head of the division of Biochemistry at the Netherlands Cancer Institute and 'bijzonder hoogleraar' at ErasmusMC (affiliated Professor). She is member of EMBO (2004), Academia Europaea (2008) and the Netherlands Academy KNAW. She received the NVBMB prize, an ERC advanced grant and several TOP research grants.

https://www.nki.nl/divisions/biochemistry/sixma-t-group/



Song Tan

Professor of Biochemistry & Molecular Biology Center for Eukaryotic Gene Regulation Penn State University University Park, PA, US

Song Tan is Professor of Biochemistry & Molecular Biology at Penn State University in Pennsylvania, U.S. He studied physics as an undergraduate at Cornell University before pursuing his Ph.D. at the MRC Laboratory of Molecular Biology in Cambridge, U.K. He continued his structural studies of transcription factor/DNA complexes as a postdoctoral fellow and project leader with Tim Richmond at the ETH-Zürich. The Tan laboratory uses biochemical and structural approaches to study how chromatin enzymes and factors interact with their nucleosome substrates.



Nicolas Thomä Friedrich Miescher Institute, Basel, Switzerland

Dr. Nicolas Thomä was educated at the University of Cambridge, UK, where he did his PhD with Dr. Peter Leadlay in enzymology, followed by postdoctoral work in structural biology with Prof. Roger Goody (Max-Planck-Institute Dortmund, Germany) and Prof. Nikola Pavletich (MSKCC, New York, USA). In 2006 Nicolas became a group leader at the Friedrich Miescher Institute in Basel, Switzerland. His lab works on the structure/function of the genome maintenance machinery, with a focus on understanding CUL4 ubiquitin ligase action and regulation. In recent years, he worked on the CRL4(CRBN) ligase and ways to modulate ligase function through small molecules.



Alessandro Vannini

Institute of Cancer Research, ICR, London, UK

Dr Alessandro Vannini studied Biology at the University of Rome "Roma Tre" and undertook his Ph.D. at IRBM in Rome. For his postdoctoral research, he joined Professor Patrick Cramer's laboratory at the LMU in Munich in 2005. In 2012, he was appointed as Team Leader in the Division of Structural Biology of the Institute of Cancer Research in London. In 2016, Dr. Vannini was elected EMBO Young Investigator, Wellcome Trust Investigator and a recipient of Programme Foundation Award from Cancer Research UK. Since 2017 he has been appointed as reader in Integrative Structural Biology at The Institute of Cancer Research.



Roger Williams, Roger L. Williams, FMedSci, FRS Group Leader MRC Laboratory of Molecular Biology Structural studies of phospholipid signalling Cambridge, UK

I am a group leader in the MRC Laboratory of Molecular Biology in Cambridge UK. Our work has focused on structural biology of the PI3K family of enzymes in signal transduction, cellular homeostasis and cancer. We use X-ray crystallography, electron cryomicroscopy and hydrogen/ deuterium exchange mass spectrometry to elucidate form and flexibility of these key protein complexes.



Wei Yang National Institutes of Health, NIH, Bethesda, US

Dr. Wei Yang is a Distinguished Investigator at the U.S. National Institutes of Health, where she has been since 1995. She was born in Shanghai, China and is a naturalized US citizen. She began her undergraduate studies of biochemistry at Fudan University in Shanghai and transferred to and received B.A. degree at SUNY at Stony Brook. She received her Ph.D. from Columbia University and postdoctoral training at Yale University. She uses a combination of structural and biochemical methods to elucidate molecular mechanisms underpinning DNA replication, repair and recombination, particularly in ATP mediated and Mg²⁺ dependent molecular recognition and catalysis.



Xiaodong Zhang Imperial College London, London, UK

Xiaodong Zhang studied Nuclear Physics in Peking University, China and obtained her PhD developing X-ray Microscopy at Stony Brook University, USA. She then went to Harvard University for her postdoctoral training in X-ray Crystallography before moving to London as a postdoctoral researcher at Imperial Cancer Research Fund. She became a lecturer at Imperial College London in 2001, promoted to Reader in 2005 and became a Professor of Macromolecular Structure and Function in 2008. Her current research focuses on using electron microscopy and X-ray crystallography to study the macromolecular complexes involved in gene regulation and DNA damage response.



CNIO - LA CAIXA BANKING FOUNDATION FRONTIERS MEETINGS | 2019

CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

Poster Session

cnio stop cancer

Molecular Mechanism of Bacterial Replicative Helicase loading

Ernesto Arias-Palomo, Puri N, O'Shea Murray VL, Yan Q, Berger JM.

¹Department of Structural & Chemical Biology, Centro de Investigaciones Biológicas,

Madrid, Spain.

²Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, US

Loading of hexameric ring-shaped helicases requires dedicated AAA+ ATPases. However, the molecular mechanisms that control nucleotide turnover and helicase loading are not well understood. We have used cryo-electron microscopy, in combination with functional assays, to analyze the helicase/loader complex from Escherichia coli. Our work provides the first high-resolution view of how replicative helicase loading occurs in bacteria and explains how this mechanism both parallels and diverges from homologous hexameric helicase and DNA polymerase clamp loader systems.

Bacillus subtilis RarA modulates replication restart

Begoña Carrasco¹, Elena M. Seco¹, María López-Sanz¹, Juan C. Alonso¹, and Silvia Ayora^{1,*}

¹Department of Microbial Biotechnology, Centro Nacional de Biotecnología, CSIC, Madrid, Spain.

The RarA/Mgs1/WRNIP protein family is highly conserved from bacteria to humans, yet its function in genome maintenance remains elusive. We show that Bacillus subtilis RarA preferentially binds singlestranded (ss) over double-stranded (ds) DNA. RarA interacts with the single-stranded binding protein, SsbA, which loads RarA onto ssDNA, and for such recruitment the amphipathic C-terminal domain of SsbA is required. RarA is a DNA-dependent ATPase strongly stimulated by ssDNA-dsDNA junctions and SsbA, or by dsDNA ends. RarA, which may interact with the replication restart protein, PriA, does not stimulate PriA DNA unwinding. In a reconstituted PriA-dependent in vitro DNA replication system, RarA inhibited initiation, but not chain elongation. The RarA effect was not observed in the absence of SsbA, or when the host-encoded preprimosome and the DNA helicase are replaced by proteins from the SPP1 bacteriophage with similar function. We propose that RarA assembles at blocked forks to delay reintiation of DNA replication and to maintain genome integrity. Through its interaction with SsbA and with a preprimosomal component, RarA might impede the assembly of the replicative DNA helicase, to control replication restart, and prevent that recombination intermediates contribute to pathological DNA replication.

The interplay between chromatin remodelers and transcription factors: Towards structural characterization of the ChAHP complex

Anja Basters, Andreas D. Schenk, Pooja Sadana, Veronika Ostapcuk, Fabio Mohn, Jan Seebacher, Marc Bühler, Nicolas H. Thomä

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

ChAHP (Chd4, Adnp, HP1) is a trimeric complex that controls the expression of lineage-specifying genes and is essential for neuronal development. The complex comprises the chromatin remodeling enzyme Chd4, the zinc finger transcription factor Adnp and the chromatin binding protein HP1β or HP1Υ. Lack of Adnp in mice is embryonically lethal. In humans, mutations in Adnp cause Helsmoortel-van der Aa syndrome - a neurological disorder that manifests with intellectual disability and also affects several other organs highlighting the essential role of Adnp in embryonic and neuronal development. Through Adnp, the ChAHP complex is recruited to euchromatic sites in the genome in a DNA sequence-specific manner. We could show that the three ChAHP proteins form a stable complex mediated by direct protein-protein interactions in vitro. Adnp represents the central part of the complex bridging the other two subunits. We mapped the interfaces between Adnp and HP1Y and Adnp and Chd4 using cross-linking mass-spectrometry and pulldowns of the recombinant proteins. In addition, we established purification of recombinant ChAHP as a homogenous monodisperse complex suited for biochemical and structural analysis.

Structural studies of MMR proteins

Doreth Bhairosing-Kok¹, Rafael Fernández-Leiro², Alexander Fish¹, Flora S. Groothuizen¹, Herrie H. Winterwerp¹, Meindert H. Lamers³, Titia K. Sixma¹

¹Division of Biochemistry and Oncode Institute, Netherlands Cancer Institute, Amsterdam, he Netherlands ²Division of structural Biology, Spanish National Cancer Research Centre (CNIO), Madrid, Spain ³Department of Chemical Immunology, Leiden University Medical Centre, Leiden, The Netherlands

DNA Mismatch Repair (MMR) is an evolutionary conserved mechanism that repairs mismatches that were incorporated during DNA replication. MMR is essential for maintaining the integrity of the genome by decreasing the number of mismatches.

MMR deficiency results in a mutator phenotype and in humans it can predispose to cancer. The first two steps in MMR in E.coli are executed by dimeric ATPases MutS and MutL. MutS scans DNA for mismatches, and upon mismatch detection, MutS undergoes an ATP-dependent conformational change and loads MutL onto the DNA. Loading of MutL causes a conformational change that allows recruitment of the next MMR proteins to initiate the repair. Here we use structural analysis of MMR proteins by X-ray crystallography and cryo electron microscopy to increase our understanding of how this transient multiprotein cascade functions. We validated our structural insights, with biophysical and enzymatic assays are presented to improve our understanding of MMR.

CNIO - LA CAIXA BANKING FOUNDATION FRONTIERS MEETINGS | 2019

Cryo-EM reveals how MutS can adapt different conformation during DNA Mismatch Repair

A. Borsellini, M. H. Lamers

Leiden University Medical Centre, Leiden, The Netherlands

MMR is a critical DNA repair system that guards the genome against errors that occur during replication. Errors in DNA repair pathways generate mutations and ultimately result in ageing and cancer in humans.

MMR involves a cascade of ATPases that read out the mismatch, and ultimately ensure the specific repair of the newly synthesized strand. This process is initiated when the dimeric MutS protein binds to a mismatch or looped out base. MutS then releases the mismatch and forms a stable ATP-dependent clamp state which can slide on DNA. The clamp state of MutS promotes the loading of MutL onto DNA which will then activate downstream effectors to nick and remove the newly synthetized strand until the mismatch is also removed. Once the mismatch is removed, the DNA replication proteins are recruited to fill the single strand DNA gap left after mismatch repair.

In my research I use Cryo Electron Microscopy together with biochemical techniques, to determine what is the driving force that brings the MutS protein to adapt to different signals in the DNA, and to undergo a series of conformational changes which start the mismatch repair cascade.

CNIO - LA CAIXA BANKING FOUNDATION FRONTIERS MEETINGS | 2019

POSTER SESSION

Template base slippage facilitates short gap repair synthesis by Prim-PoIC

Nigel C. Brissett¹, Przemysław Płociński^{1,2}, and Aidan J. Doherty¹

¹Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, UK. ²Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland

Introduction:

Prim-PolC is a bacterial repair polymerase that is orthologous to the prokaryotic NHEJ polymerase (PolDom / Prim-PolD) of the prokaryotic Ligase D complex that repairs DNA double-strand breaks in stationary phase. We previously reported that Prim-PolC interacts with core BER enzymes in vivo that constitutes an excision repair apparatus capable of repairing damaged bases and abasic sites. A notable feature of Prim-PolC is its favourable binding to short DNA gaps of 1-3 nucleotides, which it preferentially fills in with ribonucleotides. In this current study, we present structural and biochemical data that shows the relevance of a unique C-terminal extension called Loop 3, not present in Prim-PolD, that is involved in gap recognition and repair.

Methods:

In the work presented here, we used the approaches of X-ray crystallography and enzymology to determine the nature of the molecular recognition and repair synthesis of Prim-PolC on short gap DNA.

Results:

We will present data detailing the nature and strength of the interaction of Prim-PolC with short gapped DNA. High resolution X-ray crystallographic structures are also presented of Prim-PolC in ternary complexes with DNA and cofactors showing pre- and post-catalytic binding states of gap repair enzymes.

Conclusions:

These data are evidence of the very first ternary complex from a member of the proper clade of the archaeo-eukaryotic primase superfamily. The structures, and supporting biochemical evidence, provide molecular insights into the conformational steps and underlying mechanisms that Prim-PolC employs to simultaneously engage with both sides of a gap, whilst extending the 3' primer strand to fill in short gapped DNA intermediates during excision repair.

6

CDK mediated control of Yku80 in DNA repair

Maria de los Reyes Carballar Ruiz¹, Samuel Bru¹, Bàrbara Samper-Martín¹, Elisabet Ballega¹, Mariana P.C. Ribeiro¹, Josep Clotet¹, Javier Jiménez¹

¹Dpto Ciències Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Internacional de Catalunya, Barcelona, Spain

The CDK Pho85 is an enzyme intervening in specific cellular processes different to those controlled by Cdc28, the cell cycle essential CDK in Saccharomyces cerevisiae. By means of in silico experiments, we have selected different proteins that interact with Pho85. Among them we have selected for further analysis Yku80, a conserved homologue of the mammalian Ku80 protein required for telomere stability and nonhomologous end joining after DNA double strand break. We found out that Yku80 is phosphorylated both in vitro and in vivo by Pho85. A nonphosphorylatable Yku80 version shows an increase of NHEJ efficiency compared to the wild type strain. Interestingly, we found differences in cell viability between the wildtype strain and the non-phosphorylatable Yku80 when synchronized in G2 and treated with a drug that causes double strand DNA breaks, bleomycin. These differences become imperceptible if DNA damage is caused during G1. Our results suggest a possible mechanism to regulate DNA damage through the cell cycle depending on the phosphorylation state of Yku80.

In conclusion, Pho85 might phosphorylate Yku80 to control a subset of Ku heterodimer functions. Our results infer a new role of the CDK Pho85 in DNA damage and together with the studies we are performing at the moment might clarify the relevance of Pho85-Yku80 interaction. This could help to understand and describe a different process related to the cell cycle control which is the base for understanding cancer.

Bacillus subtilis DisA regulates RecA-mediated DNA strand exchange

Begoña Carrasco Cabezas, Rubén Torres, Juan Carlos Alonso

Centro Nacional de Biotecnología (CSIC), Departamento de Biotecnología Microbiana, Madrid, Spain

Bacillus subtilis diadenylate cyclase DisA converts two ATPs into c-di-AMP, but this activity is suppressed upon interaction with sites of DNA damage. DisA forms a rapid moving focus that pauses upon induction of DNA damage during spore development. DisA pausing, however, was not observed in the absence of the RecO mediator or of the RecA recombinase. suggesting that DisA binds to recombination intermediates formed by RecA in concert with RecO. We report that DisA, which physically interacts with RecA, was found to reduce its ATPase activity without competing for nucleotides or ssDNA. RecA-mediated ATP hydrolysis was inhibited by the active site DisA D77N mutation, which inactivates its DAC activity. A DisA lacking the DNA binding domain (DisA C290) also inhibited RecAmediated ATP hydrolysis. Furthermore, increasing DisA concentrations inhibit RecA-mediated DNA strand exchange, but this inhibition failed to occur when RecA was added prior to DisA, and was independent of RecAmediated nucleotide hydrolysis or increasing concentrations of c-di-AMP. We propose that DisA may preserve genome integrity by downregulating RecA activities at several steps of the DNA damage tolerance pathway, allowing time for the repair machineries to restore genome stability. DisA might reduce RecAmediated template switching by binding to a stalled or reversed fork

New Phosphorylation Sites of Rad51 by c-Met Modulates Presynaptic Filament Stability

Thomas Chabot¹, Alain Defontaine², Damien Marquis¹, Axelle Renodon-Corniere³, Emmanuelle Courtois¹, Fabrice Fleury¹, and Yvonnick Cheraud¹

¹Group of Mechanism and Regulation of DNA Repair, UFIP UMR CNRS 6286/University of Nantes, Nantes, France

²Group of Molecular Engineering and Glycobiology, UFIP UMR CNRS 6286/University of Nantes, Nantes, France

³CRCINA, INSERM, CNRS, University of Angers, University of Nantes, Nantes, France

Genomic instability through deregulation of DNA repair pathways can initiate cancer and subsequently result in resistance to chemo and radiotherapy. Understanding these biological mechanisms is therefore essential to overcome cancer. RAD51 is the central protein of the Homologous Recombination (HR) DNA repair pathway, which leads to faithful DNA repair of DSBs. The recombinase activity of RAD51 requires nucleofilament formation and is regulated by posttranslational modifications such as phosphorylation. In the last decade, studies have suggested the existence of a relationship between receptor tyrosine kinases (RTK) and DNA repair by Homologous Recombination. Among these RTK the c-MET receptor is often overexpressed or constitutively activated in many cancers and its inhibition induces the decrease of HR.

In this study, we show for the first time that c-MET is able to phosphorylate the RAD51 protein. We demonstrate in vitro that c-MET phosphorylates four tyrosine residues localized mainly in the subunit-subunit interface of RAD51. Whereas these post-translational modifications do not affect the presynaptic filament formation, they strengthen its stability against the inhibitor effect of the BRC peptide obtained from BRCA2. Taken together, these results suggest the possible role of these modifications in the regulation of the BRCA2-RAD51 interaction and underline the importance of c-MET in DNA damage response.

9

The structures of T7 bacteriophage portal and tail machinery unveil the mechanism for retention and ejection of viral DNA

Ana Cuervo^{1†}, Montserrat Fàbrega-Ferrer^{2,3†}. Cristina Machón^{2,3}, Jose Javier Conesa^{1,4}, Francisco Javier Fernández⁵, Rosa Pérez-Luque^{2,3}, Mar Pérez-Ruiz¹, Joan Pous², María Cristina Vega⁵, José López Carrascosa^{1*} and Miguel Coll^{2,3*}

¹Centro Nacional de Biotecnología, CSIC, Madrid, Spain ²Institute for Biomedical Research (IRB Barcelona), Barcelona, Spain ³Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Barcelona, Spain ⁴Present address: ALBA Synchrotron Light Source, Barcelona, Spain ⁵Centro de Investigaciones Biológicas (CIB-CSIC), Madrid, Spain

[†]These authors contributed equally to this work.

*e-mail: jlcarras@cnb.csic.es; miquel.coll@irbbarcelona.org

Viral assembly requires orchestrating a sequential and specific order of interactions among different proteins to build a mature infective particle. During the first steps of this process, bacteriophages package their genome inside an empty capsid using the energy provided by a viral ATPase (terminase). The DNA is translocated inside the capsid through the portal pore formed by the connector protein, an oligomeric ring that locates at a specific vertex of the procapsid. This protein serves also as a docking point for the rest of the tail components, which assemble after viral DNA packaging finishes. The genome can be temporarily hold inside the head in the absence of the tail components, but it remained unclear how bacteriophage proteins manage to retain highly pressured DNA after terminase departure. In mature viruses, the DNA is retained inside the ejection channel by the tail proteins, so different conformational changes need to take place to allow DNA ejection. In this work, we describe different structures of T7 bacteriophage connector and tail complex by combining cryo-EM and X-ray crystallography, which allow us to define a precise picture at the molecular level of the T7 genome retention mechanism during the assembly pathway.

Transcription-associated protein 1 (Tra1) structure revealed by single-particle cryo-EM and its role within the SAGA and NuA4 histone acetyltransferase complexes

Luis Miguel Díaz-Santín, Natasha Lukoyanova, Emir Aciyan, Alan CM Cheung

¹University College London, London, UK ²Birkbeck College, London, UK

Coactivator complexes SAGA and NuA4 stimulate transcription by posttranslationally modifying chromatin. Both complexes contain the highly conserved Tra1 subunit, a giant 3,744-residue protein from the Phosphoinositide 3-Kinaserelated kinase (PIKK) family and a direct target for multiple transcriptional activators. The full-length polypeptide was overexpressed and purified from S. cerevisiae, and an atomic model was built completely "de novo" from a 3.7 Å cryo-EM map, revealing an extensive network of alpha-helical solenoids organized into a diamond ring conformation and is strikingly reminiscent of DNA-PKcs, suggesting a direct role for Tra1 in DNA repair. The fitting of Tra1 atomic structure into existing SAGA and NuA4 cryo-EM reconstructions complements the recently available structures. Previously described mutations that affect activator targeting were mapped on Tra1 atomic model, indicating the presence of an activator interaction site within the N-terminal Finger region. The atomic structure of Tra1 is a key milestone in deciphering the mechanism of multiple coactivator complexes.

The Arabidopsis SWR1 chromatin-remodeling complex functions in DNA repair

Mattia Donà, Nicole Lettner, Ortrun Mittelsten Scheid

Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna Biocenter (VBC), Vienna, Austria

Preserving genome integrity over generations is particularly relevant in plants: the determination of the germline at a relatively late stage of development requires coping with metabolic and environmental stresses damaging DNA. DNA lesions can be repaired by several pathways, but the enzymes involved need to act even in the context of dense chromatin. The SWR1 chromatin-remodeling complex, which deposits histone variant H2A.Z, has a role in DNA repair in mammals including humans. However, mutations in SWR1 subunits are often not viable and render functionals studies difficult. In contrast, mutations in orthologous genes in Arabidopsis are less deleterious, providing a valuable experimental system to study the role of SWR1 during DNA repair. Our lab showed that loss of SWR1 complex subunits in Arabidopsis leads to impaired DNA double strand break (DSB) repair, homologous recombination and meiosis. Now we want to elucidate the detailed mechanism how the AtSWR1 complex is involved in the DSB repair process, asking for the interaction of the subunits with other proteins and the order of events during DSB repair. We used SWR1 mutant lines complemented with fusion-tagged AtSWR1 subunits PIE1, ARP6 and SWC6 for Co-IP under regular condition or after inducing DNA damage. Among the AtSWR1 potential interactors identified by MS were RUVBL1/2 homologs and TRRAP homologs, both groups of proteins with connections to DNA repair and chromatin modifiers. Taking advantage of T-DNA insertional mutants of Arabidopsis, we observed that depletion of the SWR1 subunits described above increased DNA damage sensitivity. Moreover, we generated PIE1 lossof-function mutants in different DNA repair mutants, to study the function of AtSWR1 during DNA repair. Finally, we designed inducible CRISPR/ Cas9 with gRNAs targeting different loci in eu- and heterochromatin to understand the molecular function of the AtSWR1 chromatin remodeling complex at DSB sites in a dynamic chromatin environment.

Mechanism of nucleosome assembly during DNA replication

Jan Dreyer, Clément Rouillon, Bruna Vasconcellos-Eckhardt, Francesca Mattiroli

Hubrecht Institute, Utrecht, The Netherlands

Proper inheritance of epigenetic information during cell division controls a variety of biological processes such as cell fate decisions, tissues homeostasis and development. However, we have little understanding of the underlying molecular mechanisms by which epigenetic information, specifically histone post-translational modifications on nucleosomes, are replicated in parallel to the DNA prior to cell division. In S-phase of the cell cycle, nucleosome dynamics is controlled by an interconnected network of histone chaperones that converges on the key Chromatin Assembly Factor 1 (CAF-1). CAF-1 is recruited to replication forks by the DNA polymerase processivity factor PCNA, where it assembles nucleosomes on the daughter strands during DNA replication. Recent studies have revealed the mechanism by which CAF-1 binds and deposits histones H3-H4 onto DNA in vitro. However, it remains unclear how this function is coupled to DNA replication in cells. Here, we study how PCNA controls CAF-1 activity and which molecular determinants shape their interaction. Moreover, using an *in vitro* nucleosome assembly (NAQ) assay, we can investigate the mechanisms of CAF-1 recruitment and the consequences for its activity. Future studies will focus on the effects of DNA replication stress on the PCNA-CAF-1 interaction.

This work will reveal how nucleosome assembly is controlled during DNA replication and how this is further regulated during stress situations. A mechanistic understanding of this pathway will uncover the fundamental principles that control genome and epigenome stability, thus cell fate decisions and disease avoidance.

Molecular basis for ATP-hydrolysis driven DNA translocation by the CMG helicase of the eukaryotic replisome



Patrik Eickhoff, Hazal Kose, Fabrizio Martino, Ferdos Abid Ali, Julia Locke, Andrea Nans, Hasan Yardimci and Alessandro Costa

¹Macromolecular Machines Laboratory, The Francis Crick Institute, London, UK

²Single Molecule Imaging of Genome Duplication and Maintenance Laboratory, The Francis Crick Institute, London, UK ³Structural Biology Science Technology Platform, The Francis Crick Institute, London, UK

In the eukaryotic replisome, DNA unwinding by the CMG helicase requires a hexameric ring-shaped ATPase named MCM, which spools single-stranded DNA through its central channel. The mechanism of DNA translocation is unknown, although it is clear that not all six ATPase sites around the MCM ring contribute equally to unwinding. We imaged ATP-hydrolysis driven translocation of the CMG using cryo-EM, and found that the six ATPase subunits can engage singlestranded DNA using four neighboring protomers at a time, supporting a hand-over-hand mechanism. ATP binding promotes DNA engagement, and helicase pore loops contact DNA in different modes around the MCM ring, providing a rationale for the established asymmetry in ATPase requirements for the eukaryotic replicative helicase. Not only single-stranded DNA but also fork-nexus engagement is reconfigured in different ATPase states, raising the question of whether or not translocation through the ATPase pore is efficiently coupled to fork unwinding in the isolated CMG. Imaging of a higher-order replisome assembly indicates that the Mrc1-Csm3-Tof1 (MTC) complex stabilizes the interaction between parental duplex DNA at the fork and the CMG. Because the MTC complex is known to increase the rate of replisome progression, we postulate that it might indeed function by coupling translocation and unwinding. Based on our findings and previous biochemical evidence, we propose a model for ATP-hydrolysis-driven origin DNA unwinding.

De novo modelling of R2TP-RBD domain in a cryo-em density map using Molecular Dynamics

Carlos F. Rodríguez^{1,3}, Fabrizio Martino¹, Mohinder Pal², Hugo Muñoz-Hernández^{1,3}, Laurence H. Pearl² and Oscar Llorca^{1,3}

¹Centro de Investigaciones Biológicas (CIB), Spanish National Research Council (CSIC), Madrid, Spain ²Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Falmer, Brighton, UK ³Spanish National Cancer Research Centre (CNIO), Madrid, Spain

The number of near-atomic resolution maps of protein complexes obtained using cryo electron microscopy (cryo-em) is increasing rapidly, due to availability of new microscopes, new algorithms for map reconstruction and especially new direct electron detectors. Despite the advantage of cryo-em to directly image proteins frozen in solution, however, modeling atomic structures de novo on those maps is still a difficult problem. In contrast to X-ray diffraction, usually in cryo-em the resolution is not sufficient to identify all the side chains of the protein. Computational tools can help us to model proteins on cryo-em maps at nearatomic resolution combining different techniques as secondary structure prediction, homology modeling and molecular dynamics (MD). Here we present how de novo modelling of the RPAP3 RUVBL2-binding domain (RBD) was achieved from a non-assigned density in our cryoem density map, using a set of computational tools including molecular dynamics in Amber. And also we provide some insights for the validation of the model.

The RING E3 ubiquitin ligase UHRF1 controls DNA methylation through its ability to target the maintenance DNA methyltransferase DNMT1 to newly replicated chromatin. DNMT1 recruitment relies on ubiquitylation of histone H3 by UHRF1; however, how UHRF1 deposits ubiquitin onto the histone is unknown. Here, we demonstrate that the ubiquitin-like domain (UBL) of UHRF1 is essential for RING-mediated H3 ubiquitylation. Using chemical crosslinking and mass spectrometry, biochemical assays, and recombinant chromatin substrates, we show that the UBL participates in structural rearrangements of UHRF1 upon binding to chromatin and the E2 ubiquitin conjugating enzyme UbcH5a/ UBE2D1. Similar to ubiquitin, the UBL exerts its effects through a hydrophobic patch that contacts a regulatory surface on the "backside" of the E2 to stabilize the E2-E3-chromatin complex. Our analysis of the enzymatic mechanism of UHRF1 uncovers an unexpected function of the UBL domain and defines a new role for this domain in DNMT1dependent inheritance of DNA methylation.

Critical role of the UBL domain in stimulating the E3 ubiquitin ligase activity of UHRF1 towards chromatin

Benjamin M. Foster^{1,2,3}, Paul Stolz⁴, Christopher B. Mulholland⁴, Alex Montoya², Holger Kramer², Sebastian Bultmann⁴ and Till Bartke^{1,2,3}

¹Institute of Functional Epigenetics, Helmholtz Zentrum München, Neuherberg, Germany

³Institute of Clinical Sciences (ICS), Faculty of Medicine, Imperial College London, London, UK



²MRC London Institute of Medical Sciences (LMS), London, UK

⁴Department of Biology II, Center for Integrated Protein Science Munich, Ludwig Maximilians University (LMU Munich), Munich, Germany

Holger Kramer², Sebastian Bultmann⁴ and Till Bartke^{1,2,3}

Canonical and non-canonical activities of the Holliday junction resolvase Yen1

F. Javier Aguado, Raquel Carreira, Vanesa Hurtado-Nieves and Miguel G. Blanco

Department of Biochemistry and Molecular Biology, CIMUS, Universidade de Santiago de Compostela-Instituto de Investigación Sanitaria (IDIS), Santiago de Compostela, A Coruña, Spain

Resolvases are structure-selective endonucleases (SSEs) with the ability to cleave 4-way branched DNA structures known as Holliday junctions (HJs). The paradigm of these resolvases is represented by RuvC, a bacterial resolvase that introduces two symmetrical incisions on opposite strands of HJs coordinatedly, yielding nicked DNA products that can be readily ligated. Yen1 and GEN1 are members of the Rad2/XPG-family of SSEs that were identified as the first nuclear RuvC-like resolvases in budding yeast and humans, respectively 1. Both have important roles in the removal of recombination intermediates for unhindered chromosome segregation, with their functions being tightly controlled by the cell cycle progression machinery2-4. However, while GEN1 has been extensively characterized in vitro6-7, but much less is known about Yen1 biochemistry. In this study, we have analysed the cleavage properties of Yen1 on a variety of DNA substrates. We show that while Yen1 share numerous GEN1 properties, including the range of substrates processed and the position of most of the incisions they produce, Yen1 is endowed with some differential activities: i) a robust 5'-3' exonuclease activity on nicked dsDNA that prevents the ligation of HJ resolution products; ii) the ability to remove one of the arms of a HJ structure and iii) a strong preference for cleavage of the crossing, rather the continuous strands of the HJs. Our results demonstrate the ability of Yen1 to process a variety of branched DNA intermediates that could pose a threat to chromosome segregation, but also highlight some of its potentially toxic activities that could explain the need for its stringent regulation in vivo.

Identification of Holliday junction resolvases from humans and yeast. Nature 456, 357-361.

^{1.} Ip, S.C., Rass, U., Blanco, M.G., Flynn, H.R., Skehel, J.M., and West, S.C. (2008).

Blanco, M.G., Matos, J., and West, S.C. (2014). Dual Control of Yen1 Nuclease Activity and Cellular Localization by Cdk and Cdc14 Prevents Genome Instability. Mol. Cell 54, 94–106.

Eissler, C.L., Mazón, G., Powers, B.L., Savinov, S.N., Symington, L.S., and Hall, M.C. (2014). The Cdk/Cdc14 Moule Controls Activation of the Yen1 Holliday Junction Resolvase to Promote Genome Stability. Mol. Cell 54, 80–93.

^{4.} Chan, Y.W., and West, S.C. (2014). Spatial control of the GEN1 Holliday junction resolvase ensures genome stability. Nat. Commun. 5, 4844.

Rass, U., Compton, S.A., Matos, J., Singleton, M.R., Ip, S.C., Blanco, M.G., Griffith, J.D., and West, S.C. (2010). Mechanism of Holliday junction resolution by the human GEN1 protein. Genes Dev 24, 1559–1569.

Chan, Y.W., and West, S. (2015). GEN1 promotes Holliday junction resolution by a coordinated nick and counter-nick mechanism. Nucleic Acids Res. 43, 10882–10892.
Deregulated Levels of Pontin Induce Replication Stress

Rossitsa Hristova and Anastas Gospodinov

Roumen Tsanev Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Pontin and the related ATPase Reptin participate in multiple seemingly unrelated processes such as transcriptional regulation, snoSNPs, RNAPII and telomerase assembly, DNA repair, mitosis, etc., likely functioning as chaperones to gather proteins into complexes. Pontin was shown to be essential for tumor cell growth and it is overexpressed in many cancer types. While its roles in proliferation and c-Myc dependent transcription suggest involvement in S-phase regulation its involvement in the replication process has not been directly addressed. We examined the effects of pontin deregulation on DNA replication. We found that pontin overexpression as well as its knock-down induced replication stress (RS), manifested in replication fork slow-down and accumulation of phosphorylated H2AX. In both cases, RS depended on active transcription however, the processes underlying it were different. Knock-down of pontin led to accumulation of permanently stalled ubiquitinated RNAPII complexes on chromatin, while its overexpression led to increased pause release of RNAPII. Our data indicate that changes in pontin expression increase the likelihood of replication-transcription conflicts and compromise genome integrity.

Acknowledgement: This work was supported by Bulgarian National Science Fund grant# DN 11/17 to A.G..

109

Impact of PARP1 Inhibition on Protein Dynamics in Complex DNA Lesions

Aneliya Ivanova, Aleksandrov, R., Dotchev, I., Krastev, D., Georgiev, G., Babukov, Y., Danovski, G., Dyankova, T., Atemin, A., Nedelcheva-Veleva, M., Stoynov, S.

Laboratory of Genomic Stability, Institute of Molecular Biology, Bulgarian Academy of Sciences, "Acad. G. Bonchev", Sofia, Bulgaria

Many anticancer drugs target DNA repair proteins challenging the "performance" of the repair process. While the direct effects of these drugs have been studied in detail, little is known about their systematic impact onto the dynamics of DNA repair pathways in living cells. Knowledge about this would be beneficial not only in the first steps of drug development, but also for establishing more rational and salutary therapies for cancer patients. We tested the capabilities of this approach by systematically study the effect of the anticancer drug and potent PARP1/2 inhibitor BMN673 on the kinetics of recruitment and removal of repair proteins on the sites of DNA lesions.

PARP inhibitors not only prevent the synthesis of PAR and subsequently the recruitment of PAR-dependent proteins, but they also trap PARP1 on DNA lesions. Quantification of the trapping activity of BMN673 reveals that it extends the period PARP1 spends at damage sites and PARP1 removal correlated with the recruitment of RAD51. Our results revealed that the kinetics of recruitment of the proteins participating in the early steps of DSB repair together with ubiquitin-related proteins were either unaffected or significantly less affected by PARPi compared to the kinetics of PCNA and PCNA-binding proteins, whose recruitment was considerably slowed down. As a result, RAD50, MDC1 and ATM were recruited significantly faster than PCNA and PCNA-binding proteins, which could lead to initiation of DNA resection before repair of DNA adducts near DSBs. At the same time, the error-free replication machinery was recruited simultaneously with the TLS proteins eliminating the window of opportunity for error-free DNA repair synthesis. Collectively, our results demonstrate that PARPi causes dramatic rearrangement in the order of recruitment of the repair proteins, altering the coordination and the outcome of the repair process.

110

Single-molecule insight into DNA end resection during eukaryotic dsDNA break repair

Kristina Kasaciunaite¹, Fergus Fettes¹, Maryna Levikova², Petr Cejka³ and Ralf Seidel¹

¹Peter Debye Institute for Soft Matter Physics, Universität Leipzig, Leipzig, Germany ²Institute of Molecular Cancer Research, University of Zurich, Zürich, Switzerland ³Institute for Research in Biomedicine (IRB), Università della Svizzera Italiana, Bellinzona, Switzerland

Eukaryotic dsDNA break repair by homologous recombination remains an unclear process at the molecular level. An essential step of this mechanism is the resection of the DNA 5'-end at the break. In yeast, the RecQ helicase Sgs1 and the nucleasehelicase Dna2, together with the ssDNA binding protein RPA constitute the core of the end-resection machinery. Here we study the cooperation of these proteins using magnetic tweezers. This technique allows stretching single DNA molecules and real-time monitoring of dsDNA to ssDNA conversion by a change of the DNA length. The acquired data provide information on velocity and processivity of the investigated proteins. Furthermore it allows to compare the activity of a single protein with cooperative protein groups.

In our work, Sgs1 and nuclease-dead Dna2 helicases were first characterized individually. Both proteins exhibited motor activities of DNA unwinding. Dna2 displayed processive unidirectional unwinding of long fragments and the activity of the protein was stringently dependent on RPA. On the other hand, Sgs1 repetitively opened and closed DNA self-sufficiently. In presence of RPA the processivity of unwinding increased but the rate reduced. Interestingly, the presence of RPA altered also the Sgs1 behavior during the frequent direction reversals: Sgs1 promoted DNA rezipping occurred at a similar rate as unwinding, while without RPA the DNA rezipped instantly at much higher rates.

We also succeeded to reconstitute the DNA resection process in vitro using Dna2, Sgs1 and RPA. These experiments revealed an overall slow unidirectional DNA unwinding (Dna2-like) combined with repetitive opening and closing (Sgs1-like) events. Analysis of these data showed an altered Sgs1 behavior in presence of Dna2 and suggested a direct interaction between the proteins and a formation of a ternary complex.



DNA damage caused by GC-rich cfDNA and DNA damage response in cancer cells

Ekaterina A. Kozhina¹, Nataly N. Veiko¹, Elena M. Malinovskaya¹, Vladimir P. Veiko², Larisa V. Kameneva¹, Elizaveta S. Ershova^{1,3}, Marina S. Konkova¹, Svetlana V. Kostyuk^{1,3}, Anton D. Filev^{1,4}, Ashot S. Nazaretyan^{1,5}

¹Research Centre for Medical Genetics (RCMG), Moscow, Russia

²Bach Institute of Biochemistry, Biotechnology Research Center, Russian Academy of Sciences, Moscow, Russia

³I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

⁴V. A. Negovsky Research Institute of General Reanimatology, Moscow, Russia

⁵Pirogov Russian National Research Medical University (RNRMU), Moscow, Russia

The research focused on GC-rich circulating cell-free DNA (cfDNA). There are data that its level increases several times in pathology with massive cell death (including cancer). These fragments have an increased ability to transfect into cancer cells. Moreover these fragments are able to be expressed in the cells. We have shown that GC-rich cfDNA is able to induce DNA damage in cancer cells and to launch an adaptive response to further damage. In our study, we used cancer cell cultures: MCF7 breast cancer cells and human astrocytoma cells (1321N1). The plasmid pEGFP-Gn was taken as a model for GC-rich cfDNA. On these cultures, DNA-comet assay (electrophoresis of single cells in alkaline conditions) demonstrated a rapid increase of DNA breaks rate soon (in 30 minutes) after adding pEGFP-Gn to the medium. Shortly after the maximum recorded damage degree, the number of breaks began to decrease and after 3 hours of incubation it fell down lower than the baseline (in the control). This effect was associated with a burst of reactive oxygen species after the plasmid addition, which was shown as well. In non-cancer cultures, a fast increase in DNA damage degree was not so pronounced.

In MCF7 cancer cells, DNA damage response (DDR) occured after the addition of GC-rich cfDNA, and tolerance after re-addition of GC-rich cfDNA was observed after 3 hours.

The DDR to the exposure to GC-rich cfDNA was based on upregulated expression of genes involved in DNA repair (PCNA and BRCA1) and regulation of oxidative homeostasis (HIF1A).

The data on the adaptive response to GC-rich cfDNA may be useful for the development of new approaches to the cancer therapy.

The research was supported by program of the Russian Academy of Science Presidium (No. 0517-2018-0003 under the "Basic Research for Biomedical Technologies").

112

Structure and force activation of a membrane associated complex regulating adhesion and survival upon DNA damage

Acebrón I¹, Righetto R², Bauer MS³, Redondo P¹, Boskovic J¹, Gaub HE³, Stahlberg H², **Daniel Lietha**^{1,4}

¹Spanish National Cancer Research Centre (CNIO), Madrid, Spain ²Biozentrum, University of Basel, Switzerland ³Ludwig-Maximilians-University, Munich, Germany ⁴Centro de Investigaciones Biológicas (CIB-CSIC), Madrid, Spain

Focal Adhesion (FA) complexes play key roles in cell adhesion and provide cell survival signals upon DNA damage. Importantly, FA signals allow cancer cells to survive in presence of an unstable genome which can result in resistance to DNA damaging chemo or radiotherapy, hence co-targeting of FA signals is a promising strategy to overcome resistance. FAs form initially under low force on the plasma membrane as extended complexes with a layered architecture, but are then force activated by contractile actomyosin fibers that are attached to FAs. Focal Adhesion kinase (FAK) is a key signaling component in FAs that forms the first layer closest to the membrane and is thought to be the key force sensor that converts mechanical force in FAs into a biochemical signal. The extended and dynamic nature of FAs in a membrane environment has to date precluded high resolution structural analysis, hence the molecular architecture and mechanism of force activation in FAs is currently unknown.

Employing a single particle cryo-electron microscopy approach on FAK 2D crystals formed on a lipid membrane has enabled us to obtain high resolution structural information of the first layer in FAs comprised by FAK oligomers bound to the lipid membrane. Our study reveals how FAK undergoes large conformational changes upon membrane binding and assembles into extended FAK oligomers. This represents a primed but not activated state of FAK and we propose that stretching forces in FAs can trigger full activation of FAK. To test this hypothesis we employed single molecule atomic force microscopy, enabling us to show that mechanoactivation of FAK occurs in the low pico newton range - the force range occurring natively in FAs – well below forces that result in domain unfolding.

In summary, our study provides details on the structural arrangement of the first layer in FAs formed by FAK oligomers and demonstrates how mechanical force applied on FAK molecules triggers activation of FA signals.



Circuit of phosphorylation events on histone H2A variants during DNA damage response in Arabidopsis

Anna Schmücker, Bingkun Lei, Zdravko J. Lorković, and Frédéric Berger

Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria

DNA double strand brakes (DSB) are recognized by network of conserved protein complexes which recruit and activate members of the phosphatidylinositol 3-kinase-related kinases, ATM and ATR. In eukaryotes, DNA damage response (DDR) operates in the context of chromatin consisting of repeated nucleosome structures. One of the ATM/ ATR targets, conserved from yeast to humans, is histone H2A.X which is specifically phosphorylated around the DSBs at the conserved SQ motif at the C-terminus of the protein. H2A.X is present and plays an active role in DDR in constitutive heterochromatin in fungi and animals but in the model plant Arabidopsis this domain is occupied primarily by another family of variant H2A.W, which is characterized by the presence of highly conserved KSPKK motif at the C-terminus. We found that the H2A.X specific motif SQE is also present and phosphorylated in response to DNA damage in histone variant H2A.W.7. We report that among the three H2A.W variants present in Arabidopsis, only H2A.W.6 is phosphorylated at serine in the KSPKK motif. We uncover the kinase responsible for KSPKK phosphorylation. Using genetic and synthetic approaches in Arabidopsis and fission yeast we analyze the interplay between phosphorylation at SO and KSPKK in H2A.X and H2A.W variants in the context of DNA damage response. Our data suggest that these three phosphorylation events play overlapping and distinct roles in DDR in Arabidopsis.

POSTER SESSION

Novel dithiocarbamates derivatives of 9,10-anthracenedione as potential anticancer agents



Natalia Maciejewskaa¹, Marcin Serocki¹, Marta Łapiejko¹, Maciej Bagińskia¹, Maryna Stasevych², Viktor Zvarych², Volodymyr Novikov²

¹Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Gdańsk, Poland ²Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University, Lviv, Ukraine

Malignant transformation involves a series of changes in tumor cells, that allows bypass cellular senescence. One of the principal hallmarks acquired during this process is cell immortality, achieved by maintaining constant telomeres length throughout telomerase activation. An enzyme is expressed in approximately 85% of all human cancer types but is absent in most of the somatic cells, so targeting telomerase is an attractive strategy for anticancer treatment. Inhibition of this enzyme leads to telomere erosion, or dysfunction induces a DNA damage response in cancer cells triggering apoptosis.

Anthraquinones have long been used as effective anticancer drugs against a broad spectrum of tumors. This large class of compounds has many active derivatives used successfully in anti-cancer therapy, such as anthracycline-like doxorubicin, mitoxantrone, and others. High efficacy of these drugs encourages designing new analogs with improved pharmacological properties. Thus, we present a novel dithiocarbamates of 9,10-anthracenedione with potent anticancer activity. The aim of the study was to determine the effect of new derivatives on cell cycle progression, telomerase activity, DNA damage signaling, and cytotoxic activity. Our results showed that compounds inhibit telomerase activity in a dose-dependent manner by in vitro assay, what correlates with cytotoxicity and is probably related with increased γ -H2AX foci staining, indicating a possible induction of DNA double strands breaks (DSB). This study supports the potential of new anthracenedione derivatives as a therapeutic agent for the treatment of lung cancer.

USP29: a new potential regulator of DNA Damage Tolerance through PCNA

Inés Martín-Barros, Teresa Martín-Mateos, Alain Ibáñez de Opakua, Nekane Merino, Onintza Carlevaris, Francisco J. Blanco and Edurne Berra

Structural Biology of Cancer Lab, CIC bioGUNE, Derio, Spain

The family of DeUBiquitinating enzymes (DUBs) removes mono- and poly-ubiquitin from the target proteins. DUB's activity impacts on multiple biological processes, including DNA replication. Because of their direct or indirect implication and because of their potential druggability, DUBs have become of increasing interest in recent years. USP29 is a poorly characterized member of the large family of DUBs. USP29 has been suggested as a potential oncogene as it is involved in the regulation of p53 and claspin. Furthermore, USP29 expression levels correlate with the Gleason score in prostate cancer patients. Our proteomic data showed that USP29 interacts with the DNA sliding clamp Proliferating Cell Nuclear Antigen (PCNA), which mono- or poly-ubiquitination (mUb or polyUb) plays a key role at the DNA damage Tolerance mechanisms.

We hypothesized a role for PCNA/USP29 in the cellular response to replication stress through the deubiquitination of polyUb-PCNA and/or PCNA-interacting proteins by USP29. Here, we will discuss the results of the characterization of the interaction between human USP29 and PCNA.

The Instruct Image Processing center (I2PC): support to structural biologists

Roberto Melero

CNB-CSIC, National Centre for Biotechnology (Centro Nacional de Biotecnología, CNB), Madrid, Spain

The Instruct Image Processing Center (I2PC) at the CNB-CSIC is the European Reference Center for infrastructure provision in Image Processing in Transmission Electron Microscopy and X-ray Microscopy. We provide support to structural biologists, helping them to maximize the extraction of biological knowledge from their electron microscopy images in three different platforms: Instruct, iNEXT and Corbel. In iNEXT we give support at sample level, with analysis of EM grids and acquisition of EM images using a FEI Talos Arctica and a Falcon III direct detector. In Instruct we give support for full EM image processing using SCIPION package, including movie alignment, particle picking, classification, volume reconstruction and atomic structure determination. In Corbel we link the structural data with genomics and proteomics databases, with annotations of 3D protein structures at residue level using proteomic and genomic sources including UniProt and ENSEMBL databases, diseases and genomic variants, protein domain families, disordered regions, short linear motifs and immunological epitopes.

Structural and biophysical analysis of human DNA repair protein CtIP

Davies, O.R., Forment, J. V, Sun, M., Belotserkovskaya, R., Coates, J., Galanty, Y., Demir, M., **Christopher R. Morton**, Rzechorzek, N.J., Jackson, S.P., Pellegrini, L.,

University of Cambridge, Cambridge, UK

The integrity of our genome is constantly threatened by endogenous and exogenous sources of DNA damage. The successful repair of DNA lesions is necessary for cellular survival and prevention of disease such as cancer. Therefore, complex molecular pathways have evolved to allow for accurate and timely DNA repair. It is now well-established that mammalian CtIP (CtBP-Interacting Protein) has an important function in DNA Double-Strand Break repair, by promoting the resection of DNA ends in preparation for homologous recombination (HR) and micro-homology mediated end joining (MMEJ). However, the biochemical basis of CtIP's critical role in DNA double-strand break repair remains poorly understood.

Here I describe the structural and biophysical characterisation of human CtIP and its evolutionarily conserved N-terminal domain that is critical for CtIP function. Sufficient quantities of recombinant CtIP were expressed and purified for in vitro analysis with a combination of biophysical approaches showing the presence of high molecular weight oligomeric CtIP species. The N-terminal region of CtIP has been previously shown to be essential for its role in DNA end resection, HR and MMEJ. Using X-ray crystallography in combination with small-angle X-ray scattering I demonstrate that an extended region of the CtIP N-terminus spanning amino acids 31 to 136 forms an elongated parallel helical dimer with coiled-coil segments flanking a central zinc-binding motif. In combination with previous crystallographic analysis of the N-terminal tetramerisation motif of CtIP (Davies et al., 2015), my new structural information allows modelling the overall architecture of the CtIP NTD.

Structural basis for DNA recognition by human RECQ4 DNA helicase



Anna C. Papageorgiou¹, Lumir Krejci², and Konstantinos Tripsianes¹

¹CEITEC-Central European Institute of Technology, Masaryk University, Brno, Czech Republic ²National Centre for Biomolecular Research and Department of Biology, Masaryk University, Brno, Czech Republic

RECQ4 is a member of the RecQ helicase family, an evolutionarily conserved class of ubiquitous enzymes involved in maintaining chromosomal stability. RECQ4 plays a role at the intersection of telomere maintenance, DNA damage response and replication although the exact mechanism of function for this enzyme is not yet known. Using NMR and other biophysical methods, we study the mostly disordered N-terminus region of RECQ4. RECQ4 contains three DNA-binding regions and during its interaction with DNA it remains in a disordered state (fuzzy complexes). We have identified specific residues implicated in binding of diverse DNA structures, such as single-stranded, double-stranded, Y-form, Holliday junction, or G-quadruplex, in a salt-dependent manner. The electrostatic component is of prime importance to the interactions, yet the presence of a junction further stabilizes the complexes. Future structural studies aim to understand in detail the DNA-binding mode and the underlying functional plasticity.

Smc1-ser360-phosphorylation as a biodosimetry-biomarker in Human Peripheral Blood Mononuclear cells (PBMCs) via Radiation-induced activation of chromatin protein

Eun-Young Park, Kang Hyun Kim, Je-won Ryu, Hyoun Sik Kim, Sang-Wook Lee

¹Department of Radiation Oncology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea ²Department of Convergence Medical Research Center, Asan Medical Center, Seoul, Korea

BACKGROUND: After the event of a mass radiological incident, there is an urgent need for fast and potential identify individual exposures for triaging and providing dose-appropriate medical intervention.

AIM: This study aimed to develop a new biodosimetry method using SMC1 phosphorylation as a measure of exposure to radiation. Structural maintenance of chromosomes 1 (SMC1) is phosphorylated in response to ionizing radiation.

EXPERIMENT & RESULTS: After irradiation (0-10 Gy, using X-ray irradiator), the levels of SMC1 phosphorylation at Ser-360 and Ser-957 were assessed using Western blotting in normal human cell lines (WI-38VA-13, HaCaT) and four GM cells (lymphoblastoid cell lines from normal human blood). Subsequently, we investigated that the quantification of radiation induced-SMC1 activation using peripheral blood mononuclear cells (PBMCs) obtained from 20 healthy adults through clinical trials. Phosphorylation of SMC1 at Ser-957 and Ser-360 was increased by exposure (0-4Gy, 6MV proton produced by medical linear accelerator) in a dose-dependent manner, peaked at 1–3 h postirradiation and then decreased gradually. Ser360 was identified as a new phosphorylation site and was more sensitive to radiation than Ser-957, especially at doses below 1 Gy. Our results demonstrate a robust ex vivo response of phospho-SMC1-(Ser-360) to ionizing radiation in human PBMCs.

CONCLUSION: Detection of phosphorylation at Ser-360 in SMC1 could be used as a biodosimetry biomarker of radiation exposure. Our findings suggest that it is feasible to measure blood cell-based changes in the phosphorylation level of a protein, even after low-dose exposure.

Bypass of ribonucleotides by DNA Pol ε

Vimal Parkash, Pia Osterman and Erik Johansson

Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden

DNA polymerase ε , a eukaryotic multi-subunit B-family polymerase, frequently incorporates ribonucleotides¹⁻³, and failure to remove them results in replication stress caused by stalling during bypass of ribonucleotides in the DNA template4. Previous biochemical and structural studies of the bacteriophage RB69 gp43 DNA polymerase while encountering ribonucleotides at different positions in the DNA template showed that increased stalling during ribonucleotide bypass is associated with displacement of Y391 (corresponds to Y553 in Pol ε)1. Here we present a series of structures of yeast replicative DNA polymerase ε capturing the bypass of a ribonucleotide in the DNA template.

References

- Clausen, A.R., Murray, M.S., Passer, A.R., Pedersen, L.C. & Kunkel, T.A. Structurefunction analysis of ribonucleotide bypass by B family DNA replicases. *Proc Natl Acad Sci U S A* 110, 16802-7 (2013).
- Nick McElhinny, S.A. et al. Genome instability due to ribonucleotide incorporation into DNA. Nat Chem Biol 6, 774-81 (2010).
- Nick McElhinny, S.A. et al. Abundant ribonucleotide incorporation into DNA by yeast replicative polymerases. *Proc Natl Acad Sci U S A* 107, 4949-54 (2010).
- Watt, D.L., Johansson, E., Burgers, P.M. & Kunkel, T.A. Replication of ribonucleotidecontaining DNA templates by yeast replicative polymerases. DNA Repair (Amst) 10, 897-902 (2011).

POSTER SESSION

The first BRCT domain of XRCC1 mediates binding to both poly(ADP-ribose) and DNA

Luis Mariano Polo¹, Yingqi Xu², Peter Hornyak¹, Zihong Zeng¹, Steve J. Matthews², Keith W. Caldecott¹, Antony W. Oliver¹ and Laurence H. Pearl¹

¹Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Falmer, Brighto, UK

²Cross-Faculty NMR centre, Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, London, UK

XRCC1 controls repair of single-strand DNA breaks by acting as a scaffold protein for the recruitment of Polβ, LigIIIα and end-processing factors such as PNKP and APTX. XRCC1 itself is recruited to DNA damage through the interaction of its central BRCT domain with poly-(ADP-ribose) (PAR) chains generated by PARP1 or PARP2. Although it has long been believed that XRCC1 itself binds DNA, there was no precise data about its possible biological relevance. By using NMR, biochemistry, biophysics and cell biology, we show BRCT domain simultaneously mediates interaction of XRCC1 with poly-(ADP-ribose) and DNA, through separate and non-overlapping binding sites on opposite faces of the domain. Mutation of residues involved in this interaction, which includes the site of a common disease-associated human polymorphism, affects DNA binding of this XRCC1 domain *in vitro*, but do not affect PAR binding. Its consequence is an impairment of XRCC1 recruitment and retention at DNA damage and repair of single-strand breaks *in vivo*.

122

POSTER SESSION

Particulate Matter (PM10) alters the Nucleotide Excision Repair pathway in lung cells



Ericka Marel Quezada Maldonado^{1,4}, Yesennia Sánchez Pérez¹, Yolanda Irasema Chirino², María Eugenia Gonsebatt Bonaparte³ and Claudia María García Cuellar¹

¹Subdirección de Investigación Básica. Instituto Nacional de Cancerología

²Unidad de Biomedicina. Facultad de Estudios Superiores Iztacala, UNAM

⁴Programa de Doctorado en Ciencias Biomédicas, UNAM. Subdirección de Investigación Básica.

Instituto Nacional de Cancerología. Tlalpan D.F., México

Particulate matter with a mean aerodynamic diameter $\leq 10 \mu m$ (PM10) is one of the main air pollutants and contains metals, polycyclic aromatic hydrocarbons (PAH) and biological elements. According to IARC, is classified as carcinogen for humans, mainly associated with lung cancer. PM10 generates inflammation, oxidative stress and DNA damage specifically double strand breaks and adducts. Although PM10 increases expression of proteins responsible for damage recognition such as ATM and p53, it can also decrease the efficiency of DNA repair systems, particularly of NER pathway, which eliminates adducts of PAHs, however the mechanisms behind this are unknown. NER involves recognition, verification and elimination of DNA damage stages through XPC, RAD23, XPD, XPA and ERCC1 and deregulation in components of this system can promote accumulation of DNA lesions. Objective: To evaluate if PM10 induce alterations in gene expression, protein level and posttranslational modifications NER components in a time-dependent manner, and if changes have an impact on the repair of DNA. Results: The exposure of epithelial lung cells (A549) to 10 µg/cm2, generated an increase in protein levels of RAD23 (6h), downregulated gene expression of ERCC2 (6h-24h), increased XPD protein levels (12-24h), and also upregulated XPA gene expression (12-48h) decreasing XPA protein levels (24h). Besides PM diminished phosphorylation of XPA after 24h of exposure. Conclusions: Genomic instability is a hallmark of cancer that occurs when DNA cannot be adequately repaired. In this work we found that PM10 deregulated the expression of ERCC2 and XPA genes, also decreased the level protein and phosphorylation of XPA. Because this protein is a rate-limiting factor for the NER process, and its expression level determines NER capacity, these alterations could modify the formation and DNA coupling of protein complexes necessary for the elimination of DNA damage through the NER pathway after exposure to PM10.

³Instituto de Investigaciones Biomédicas, UNAM

Exoxome and Exosomal miRNA as Predictive Radiotherapy Biomarkers and Therapeutic targets

Je-won Ryu, Eun-Young Park, Hyoun Sik Kim, Kang Hyun Kim, Sang-Wook Lee

¹Department of Radiation Oncology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea ²Department of Convergence Medical Research Center, Asan Medical Center, Seoul, Korea

BACKGROUND: Injury of normal tissues during Radio-therapy (RT) is a contemporary clinical problem. Despite in greatly developing RT techniques, translation of RT into effective personalized clinical methods is hindered by the absences of reliable biomarkers. Unirradiated tissues effect as a result of signaling received from nearby irradiated cells, bystander effects, which lead to damaged normal tissues including DNA damage, repairing failure, or abnormal immune response. Exosomes or various vesicles are released by tumor and normal tissues to nearby and distinct environments.

AIM: Exosome between radiation-damaged cells with their environments may play a role in intensified the damage. This study aimed to elucidate how exosomal miRNAs regulate protein expression to induce normal cell damage and to resist cancer cell death.

EXPERIMENT & RESULTS: We investigated whether the exosome from cancer and normal cells exposed to radiation may change in size or in number than normal conditioned cells. Dynamic light scattering (DLS) analysis showed that the size and number of exosomes from 0-8Gy RT-exposed cells were critical increased with radiation-dose dependently. Here, we analyzed the exosomal miRNA of 4, 8Gy RTexposed A549 lung cancer and WI38 epidermal normal cells. The expression of their exosomal miRNA was showed different profiles. MicroRNA expression is frequently dysregulated in damaged normal tissues and may be reflected by distinct exosomal miRNA profiles isolated from RT-exposed tissues. In our investigation, immunoblotting showed that inflammatory CRP expression in normal condition cells was increased the effects of only exosome from RT-exposed cells.

CONCLUSION: We can conclude that characterization of exosome and exosomal miRNA are very important steps towards better understanding of predictive or estimated treatment effect in cancer patient with radiotherapy.

Structural and molecular biology of the DNA damage response

53BP1, RIF1 and the SHIELDIN complex in DNA repair pathway choice



Pooja Sadana¹, Andreas Daniel Schenk¹, Dheva Setiaputra³, Alexandra Graff Meyer¹, Julia Reinert¹, Daniel Durocher², Nicolas H Thomä¹

¹Friedrich Miescher Institute for Biomedical Resarch, Basel, Switzerland ²Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

Double-strand breaks (DSBs) are one of the most cytotoxic forms of DNA lesion. If not repaired, they cause genomic rearrangements and cell death. In cells, DSBs are mainly repaired by two major pathways - homologous recombination (HR) and non-homologous end joining (NHEJ). HR involves 5' end resection followed by strand invasion, and the new strand is synthesized using the intact DNA strand as a template, whereas in NHEJ, both ends are joined without further processing. The choice between NHEJ and HR pathways is mainly determined by 53BP1 (Tumor suppressor p53 binding protein1). It is known that 53BP1 favors NHEJ by suppressing 5' end resection. But the exact mechanism by which 53BP1 blocks the resection nucleases is not known. Recently published papers showed that the 53BP1-RIF1 complex recruits SHIELDIN (SHLD1, SHLD2, SHLD3, REV7) that blocks end resection, and favors NHEJ.

In this study, we aim to solve the high-resolution structure of the RIF1-SHIELDIN complex in an effort to understand the structural basis of molecular mechanisms underlying the DNA damage response. To achieve this, we overexpressed RIF1-SHLD3-REV7 subunits in insect cells. The resulting complex was purified by affinity and size-exclusion chromatography to homogeneity. We further solved the lowresolution negative stain model of the complex, which clearly shows the envelopes for both RIF1, and SHLD3-REV7. Currently, we are in the process of optimizing grid-freezing conditions to solve the high-resolution structure by cryo-electron microscopy.

35

Structural insights into the function of ZRANB3 in replication stress response

Marek Sebesta¹, Christopher D. O. Cooper², Antonio Ariza, Christopher J. Carnie, Dragana Ahel

¹Present address: Centre for Structural Biology, Central European Institute of Technology, Masaryk University, Brno, Czechia

²Present address: Department of Biological Sciences, University of Huddersfield, Huddersfield, UK

DNA replication is essential prerequisite for successful cell division. Its accurate and timely execution is crucial for maintenance of genome stability. At the same time, the integrity of DNA is constantly challenged by endogenous as well as exogenous factors that may impinge on the progression of DNA replication by stalling the replication forks. An intricate network of pathways, collectively known as DNA damage tolerance (DDT), operates to restart the stalled replication forks. The choice of a particular pathway is governed by PCNA and its posttranslational modifications, mainly (poly)ubiquitination. PCNA is the key replication factor that recruits a diverse array of factors required for both replication and DDT. Polyubiquitination of PCNA is triggered at sites where replication forks stall. ZRANB3 is the only vertebrate factor known to be recruited these sites. ZRANB3 can act in two modes: it may remodel the stalled replication forks thereby promoting bypass of DNA damage, or it may initiate DNA repair by creating a nick on the leading strand ahead of the replication fork. The molecular mechanism of the latter mode is poorly understood. We therefore focused our interest on understanding how ZRANB3 nicks DNA using its nuclease domain of HNH type. We determined the structure of the HNH domain and performed a detailed mutational analysis. Additionally, we studied the role that PCNA has in regulating the nuclease activity both at a functional and structural level. Interestingly, we found that the HNH domain of ZRANB3 contains a unique insertion that is essential for its activity. Moreover, we found out that PCNA stimulates the nuclease activity of ZRANB3 via a direct protein-protein interaction. Taken together, our data indicate that PCNA first recruits ZRANB3 to its site of action, but later plays a distinct role in promoting its nuclease activity. Importantly, we reveal that a subset of cancerassociated mutations in ZRANB3 abolish its nuclease activity.

Synthetic lethal strategies for cancer treatment in Homologous Recombination-deficient models

Laura Sesma-Sanz, Nadine Brahiti, Amélie Rodrigue, Jean-Yves Masson

Genome Stability Laboratory, Laval University Cancer Research Center, Hôtel-Dieu de Québec, Quebec City (Qc), Canada

The idea of synthetic lethality, where the combination of two genetic events results in the death of the cell, has acquired significant importance in the recent efforts to design new cancer therapies. BRCA1, PALB2 and BRCA2 are cancer susceptibility genes that code for proteins involved in the Homologous Recombination (HR) pathway of DNA Repair. Targeting other DNA Repair pathways in cancer cells deficient for any of these factors is one of the main lines of research in cancer applied synthetic lethality. For example, inhibition of PARP-1, a DNA Repair protein, was observed to be synthetically lethal with HR deficiency. However, many patients develop a resistance to PARP inhibitors, therefore justifying the need for alternative therapies. We have developed a new cellular strategy to find synthetic lethal interactions by testing compound libraries on an HR-deficient cellular environment. We have completed the screening using the LOPAC1280 library, a set of 1280 compounds that includes already approved drugs, on a BRCA2-deficient fluorescent cell line. This optimized color-based method will invert the traditional screening direction by starting with the drugs and then finding the biological target. We are currently testing one of the top compounds and aim to understand its mechanism of action. These drugs could potentially be used in clinical trials for the treatment of different types of HR-deficient cancer, as an alternative or combined with already existing treatments, considering there are not many specific therapies for these cases. The discovery of new targets could be the base for new compound development studies. The use of fluorescent proteins for the screening of molecules in a cellular system can also be extended to the study of other disease models.

Protein Dynamics in complex DNA lesions

Stoyno Stoynov, Aleksandrov, R., Dotchev, I., Krastev, D., Georgiev, G., Babukov, Y., Danovski, G., Dyankova, T., Ivanova, A., Atemin, A., Nedelcheva-Veleva, M.

Institute of Molecular Biology, Bulgarian Academy of Sciences, "Acad. G. Bonchev", Sofia, Bulgaria

A single mutagen can generate multiple different types of DNA lesions. How different repair pathways cooperate in complex DNA lesions, however, remains largely unclear. Here, we measured, clustered and modeled the kinetics of recruitment and dissociation of 70 DNA repair proteins to laser-induced DNA damage sites in HeLa cells. The precise timescale of protein recruitment reveals that error-prone translesion polymerases are considerably delayed compared to error-free polymerases. We show that this is ensured by the delayed recruitment of RAD18 to double-strand break sites. The time benefit of error-free polymerases disappears when PARP inhibition significantly delays PCNA recruitment. Moreover, removal of PCNA from complex DNA damage sites correlates with RPA loading during 5'-DNA end resection. Our systematic study of the dynamics of DNA repair proteins in complex DNA lesions reveals the multifaceted coordination between the repair pathways and provides a kinetics-based resource to study genomic instability and anticancer drug impact.

Fe-S cluster biogenesis in DNA repair proteins: Structural studies of the CIA machinery

Susanne Kassube, Nicolas Thomä

Friedrich Miescher Institute for Biomedical Research, University of Basel, Basel, Switzerland

Fe-S clusters are ancient and versatile protein cofactors that are found in all three domains of life. A striking number of proteins involved in DNA replication and repair contain Fe-S clusters: DNA polymerases and DNA primase, as well as DNA helicases. In eukaryotes, the cytosolic iron sulfur assembly (CIA) pathway mediates the insertion of Fe-S clusters into these proteins. A critical component of this pathway is the CIA targeting complex that recognizes client proteins and facilitates Fe-S cluster transfer. To shed light on the molecular mechanism of Fe-S cluster transfer, we determined crystal structures of a heterodimeric catalytic core of the CIA complex, and of the entire CIA targeting complex that additionally contains MMS19, the adaptor molecule required for the recruitment of DNA repair and replication proteins.

The structures reveal evolutionarily conserved surface features of the complex involved in client protein recruitment. Cryo-electron microscopic studies of the CIA targeting complex bound to different client proteins, together with mutational analysis, *in vitro* binding studies, and yeast complementation assays, confirm these as bona fide interaction sites. Combined, these results highlight the high evolutionary conservation of the CIA pathway from yeast to human.

Our structural analysis of the CIA targeting complex visualizes a key component of the CIA pathway, providing a framework for the interpretation of previous biochemical and cell biological data. This work thus contributes towards our mechanistic understanding of Fe-S cluster biogenesis, and sheds further light on the critical biological functions of Fe-S clusters in DNA repair and replication factors.

Bacillus subtilis RecA interacts with and loads RadA/Sms to promote branch migration during natural chromosomal transformation and DNA repair

Rubén Torres Sánchez, Ester Serrano, Juan Carlos Alonso

Centro Nacional de Biotecnología (CSIC), Departamento de Biotecnología Microbiana, Madrid, Spain

Background:

RadA protein, that has a RecA-like ATPase domain, is conserved from bacteria to plants, but its role is poorly understood. Streptococcus pneumoniae RadA is a 5'-->3' DNA helicase involved in natural chromosomal transformation. Deletion of Escherichia coli radA renders cells marginally sensitive to DNA damaging agents. E.coli RadA acts as a branch migration translocase during RecA-catalysed strand exchange reaction, but no helicase activity has been documented.

Objectives:

The main objective is to characterize B.subtilis RadA/Sms, together with the recombinase RecA, in natural transformation and DNA repair, processes that play a central role in bacterial evolution, survival and antibiotic resistance genes spreading.

Methods:

In vivo and *in vitro* approaches were used: determination of transformation efficiency and sensitivity to DNA damaging agents in the absence of radA and in different RadA/Sms mutant variants in its Zn-binding (C13A, C13R) and Walker A (K104R) domains; and characterization of its interaction with other proteins, ATPase, DNA binding and helicase activities of RadA/Sms and its variants, in concert with RecA.

Results:

Single-stranded (ss)DNA stimulates the ATPase activity of C13A and C13R, but not of wt RadA/Sms. wt and K104R, but not C13A and C13R RadA/Sms, inhibit ssDNA-bound RecA-mediated ATP hydrolysis. wt and C13A RadA/Sms unwind a 5'-tailed DNA in the 5'-->3' direction. In the presence of RecA, wt RadA/Sms also unwinds a 3'-tailed DNA, but not C13A RadA/Sms, that cannot interact with RecA. We propose that RecA interacts with and recruits RadA/Sms onto ssDNA to facilitate D-loop extension in the 5'-->3' direction, promoting chromosomal transformation and DNA repair.



Why does UvrD interact with RNA polymerase?



Iñigo Urrutia Irazabal¹, Frank Sobott², Nigel J. Savery¹ and Mark S. Dillingham¹

¹School of Biochemistry, Medical Sciences Building, University of Bristol, Bristol, UK ²Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK

Transcription-coupled repair (TCR) is a sub-pathway of nucleotideexcision repair involved in repairing bulky lesions on the transcribed strand of DNA. When RNA polymerase (RNAP) detects a DNA lesion, it stalls and recruits Mfd. Mfd pushes the RNAP forward and recruits repair machinery to the newly-exposed lesion. Recently, another TCR pathway has been proposed where, instead of Mfd, UvrD is recruited, along with the pausing factor NusA, and promotes backtracking of the RNAP to initiate repair. UvrD is meant to dimerise while interacting with RNAP due to its increased expression during the stringent response. However, although there is clear evidence for a physical interaction between RNAP and UvrD, there is still considerable controversy about the exact function of this interaction. Here, we present a structural analysis of the Bacillus subtilis RNAP transcription elongation complex (TEC) and its interactions with PcrA (the UvrD orthologue in B. subtilis). The RNA:DNA scaffold that allows TEC formation significantly increases the affinity of PcrA for RNAP, suggesting that PcrA binds to a particular conformation and engages with nucleic acid that emerges from the TEC. From in vitro biochemical analysis and ex vivo pulldown-mass spectrometry analysis we have not found any evidence suggesting a ternary complex with NusA. However, our biochemical data supports the pro backtracking role of PcrA on RNAP.

Dynamics of ATM, ATR and their substrates in Complex DNA Lesions

Sonya Uzunova, Aleksandrov, R., Dotchev, I., Krastev, D., Georgiev, G., Babukov, Y., Danovski, G., Dyankova, T., Ivanova, A., Atemin, A., Nedelcheva-Veleva, M., Stoynov, S.

¹Institute of Molecular Biology, Bulgarian Academy of Sciences, "Acad. G. Bonchev", Sofia, Bulgaria ²Laboratory of Genomic Stability, Institute of Molecular Biology, Bulgarian Academy of Sciences, "Acad. G. Bonchev", Sofia, Bulgaria

Ataxia Telangiectasia Mutated (ATM), Ataxia Telangiectasia and Rad3 related (ATR) and DNA-dependent protein kinase (DNA-PK) are the major PIKK protein kinases that act in the cellular response to DNA damage by phosphorylating serine/threonine residues in a large number of proteins. ATM and DNA-PK were found to be recruited to doublestranded breaks, whereas ATR is recruited by binding to single-stranded DNA coated with RPA proteins. Once recruited to DNA lesions PIKK protein kinases interact and phosphorylate dozens of proteins to orchestrate the checkpoint control and the maintenance of genomic integrity. However, little is known about the order and the kinetics of recruitment and removal of these kinases to the sites of complex DNA damages with respect to the dynamics of their substrates. Here, we measured, clustered and modeled the kinetics of recruitment and dissociation of ATM and 70 DNA repair proteins to laser-induced DNA damage sites in HeLa cells. Our results reveal two consecutive waves of recruitment of PIKK protein kinases and determine their precise coexistence with most of their substrate proteins shedding light on unknown aspects of the repair of complex DNA lesions.

Elucidation of the ATR/ATRIP activation mechanism

Melanie Weisser, Diana Kowalik, Guillermo Montoya

NNF Centre for Protein Research, University of Copenhagen, Copenhagen, Denmark

To avoid undesired genome modifications, the cell has developed a sophisticated DNA damage response (DDR), which detects and repairs DNA damage. The ATM and RAD3-related (ATR) protein kinase is one of the master regulators of the DDR controlling cell-cycle transitions, DNA replication, DNA repair and apoptosis [1]. Despite our understanding of ATR functions in maintaining genome integrity, the molecular details of the complex mechanisms involved in its activation are not yet understood. Recent functional studies by our collaborator group and others have identified the RPA complex, TopBP1 and ETAA1 as factors that are critical for the activation of ATR signalling and involved in the recruitment of ATR and its obligate co-factor ATRIP (ATR interacting protein) to sites of DNA damage [2,3]. In our lab, we have managed to establish the recombinant co-expression and purification of the human ATR-ATRIP complex from mammalian cells as well as the in vitro reconstitution of the heterotrimeric RPA complex, which has allowed us to approach the structural and functional investigation of the interactions between the ATR-ATRIP complex and its activators.

Through an integrative approach that combines high-resolution cryoelectron microscopy (cryo-EM) with single-molecule spectroscopy studies and cell-based kinase activity assays we aim to dissect the molecular mechanisms of ATR-ATRIP activation by RPA, TopBP1 and ETAA1 and to decipher how ATR-ATRIP and its binding partners safeguard the faithful duplication of the genome.

^[1] Cortez, D. et al., Science 294, 1713-1716 (2001).

^[2] Kumagai, A. et al., Cell 124, 943-955, (2006).

^[3] Haahr, P. et al., Nat Cell Biol 18, 1196-1207, (2016).



CNIO - LA CAIXA BANKING FOUNDATION FRONTIERS MEETINGS | 2019

CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20 – 22 May 2019

Structural and molecular biology of the DNA damage response

Previous CNIO Frontiers Meetings and CNIO Cancer Conferences

cnio stop cancer

2018

MOLECULAR, CELLULAR AND ORGANISMAL HALLMARKS OF AGING

07/05/2018 – 09/05/2018 Organisers: Maria A. Blasco, Alejo Efeyan, Kathleen Collins, Thomas Rando

FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY

09/07/2018 – 11/07/2018 Organisers: Victoria Aranda, Nabil Djouder, Joao Monteiro, Marisol Soengas, Laurence Zitvogel

2017

PRIMARY AND SECONDARY BRAIN TUMORS

19/02/2017 - 22/02/2017

Organisers: Massimo Squatrito, Manuel Valiente, Richard Gilbertson, Michael Weller

MOLECULAR CHAPERONES IN CANCER

02/05/2017 – 04/05/2017 Organisers: Nabil Djouder, Wilhelm Krek, Paul Workman, Xiaohong Helena Yang

2016

CANCEROMATICS III – TUMOR HETEROGENEITY

13/11/2016 – 16/11/2016 **Organisers:** Fátima Al-Shahrour, Núria Malats, Alfonso Valencia, Chris Sander

2015

METASTASIS INITIATION: MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES 28/09/2015 - 30/09/2015 Organisers: David Lyden, Yibin Kang, Gemma Alderton, Victoria Aranda, Li-kuo Su, Héctor Peinado

NEW TRENDS IN ANTICANCER DRUG DEVELOPMENT 22/03/2015 - 25/03/2015

Organisers: Manuel Hidalgo, Alberto Bardelli, Lillian Siu, Josep Tabernero

2013

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER 27/05/2013 - 29/05/2013 Organisers: Robert Benezra, Ana Losada, Marcos Malumbres, René Medema

2012

ALLOSTERIC REGULATION OF CELL SIGNALLING

17/09/2012 - 19/09/2012 Organisers: Francesco Gervasio, Ermanno Gherardi, Daniel Lietha, Giulio Superti-Furga

2011

RECAPTURING PLURIPOTENCY: LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER

07/11/2011 - 09/11/2011

Organisers: Maria A. Blasco, Konrad Hochedlinger, Manuel Serrano, Inder Verma

CANCEROMATICS II : MULTILEVEL INTERPRETATION OF CANCER GENOME

28/03/2011 - 30/03/2011

Organisers: Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

BREAST CANCER

07/02/2011 - 09/02/2011

Organisers: Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

2010

CANCER PHARMACOGENETICS: PERSONALIZING MEDICINE

22/11/2010 - 24/11/2010

Organisers: Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

MOLECULAR CANCER THERAPEUTICS

08/03/2010 - 10/03/2010 Organisers: Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

2009

THE ENERGY OF CANCER

02/11/2009 - 04/11/2009 Organisers: Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

CANCER-OM-ATICS : MULTILEVEL INTERPRETATION OF CANCER GENOME DATA

06/07/2009 - 08/07/2009

Organisers: Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

STEM CELLS AND CANCER

23/02/2009 - 25/02/2009 Organisers: Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

2008

SIGNALLING UPSTREAM OF mTOR

03/11/2008 - 05/11/2008 Organisers: Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sánchez-Céspedes

STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL

23/06/2008 - 25/06/2008

Organisers: Niko Grigorieff, Eva Nogales and Jose María Valpuesta

DEVELOPMENT AND CANCER

04/02/2008 - 06/02/2008

Organisers: Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

2007

LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY

05/11/2007 - 07/11/2007

Organisers: Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS

11/06/2007 - 13/06/2007

Organisers: Robert N. Eisenman, Martin Eilers and Javier León

MOLECULAR MECHANISMS IN LYMPHOID NEOPLASM

19/02/2007 - 21/02/2007 Organisers: Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

2006

TELOMERES AND TELOMERASE-CNIO / JOSEF STEINER CANCER CONFERENCE

13/11/2006 - 15/11/2006 Organisers: Maria A. Blasco and Jerry Shay

MEDICINAL CHEMISTRY IN ONCOLOGY

02/10/2006 - 04/10/2006 Organisers: Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

INFLAMMATION AND CANCER

22/05/2006 - 24/05/2006 Organisers: Curtis Harris, Raymand Dubois, Jorge Moscat and Manuel Serrano

PTEN AND THE AKT ROUTE

08/05/2006 - 10/05/2006

Organisers: Ana Carrera, Pier Paolo Pandolfi and Peter Vogt

2005

CANCER AND AGING

07/11/2005 - 09/11/2005

Organisers: Maria A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano

MAP KINASES AND CANCER

30/05/2005 - 01/06/2005 Organisers: Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda

ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS

07/03/2005 - 09/03/2005 Organisers: Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks

2004

CADHERINS, CATENINS AND CANCER

29/11/2004 - 01/12/2004 Organisers: Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

STRUCTURAL BIOLOGY OF CANCER TARGETS

27/09/2004 - 29/09/2004 Organisers: Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

2003

APOPTOSIS AND CANCER

01/12/2003 - 03/12/2003 Organisers: Gabriel Nuñez, Marisol Soengas and Scott Lowe

SMALL GTPases IN HUMAN CARCINOGENESIS

16/06/2003 - 18/06/2003

Organisers: Juan Carlos Lacal, Channing Der and Shuh Narumiya

TARGETED SEARCH FOR ANTICANCER DRUGS

17/03/2003 - 19/03/2003

Organisers: Amancio Carnero and David H. Beach

2002

MECHANISMS OF INVASION AND METASTASIS

18/11/2002 - 20/11/2002 Organisers: Joan Massagué and Richard Hynes

THE CELL CYCLE AND CANCER

30/09/2002 - 02/10/2002

Organisers: Marcos Malumbres, Charles Sherr and Jiri Bartek

CANCER EPIGENETICS : DNA METHYLATION AND CHROMATIN

29/05/2002 - 31/05/2002

Organisers: Manel Esteller and Stephen B. Baylin

142

FRONTIERS MEETINGS 2019

Madrid 20 - 22 May 2019

Abstract Submission deadline 22 April Application deadline 6 May

Structural and molecular biology of the DNA damage response

Organisers

Spain

Spain

Oscar Llorca

Spanish National Cancer Research Centre

Rafael Fernández Leiro

Laurence H. Pearl

Titia Sixma

Netherlands

Genome Damage and Stability Centre-University of Sussex, UK

Netherlands Cancer Institute, NKI,

Spanish National Cancer Research Centre.

Confirmed Speakers

James Berger Johns Hopkins School of Medicine, USA

Maria A. Blasco Spanish National Cancer Research Centre, Spain

Alessandro Costa, The Francis Crick Institute, UK

Patrick Cramer Max Planck Institute for Biophysical Chemistry Germany

Aidan Dohertv Genome Damage and Stability Centre, University of Sussex, UK

Daniel Durocher The Lunenfeld-Tanenbaum Research Institute, Canada

Karl-Peter Hopfner Gene Center Munich. Germany

MINISTERIC DE CENCI

iE

Meindert H. Lamers Leiden University Medical Center, Netherlands

Oscar Llorca Spanish National Cancer Research Centre, Spain

Juan Méndez Spanish National Cancer Research Centre, Spain

Eva Nogales Lawrence Berkeley National Laboratory, Howard Hughes Medical Institute, USA

Lori Passmore Laboratory of Molecular Biology, MRC-LMB, UK

Laurence H. Pearl Genome Damage and Stability Centre-University of Sussex, UK

Luca Pellegrini University of Cambridge, UK

Song Tan Penn State University, USA

Nicolas Thomä Friedrich Miescher Institute, Switzerland

Alessandro Vannini Institute of Cancer Research ICR. UK

Roger Williams Laboratory of Molecular Biology, MRC-LMB, UK

Wei Yang National Institutes of Health, NIH USA

Xiaodong Zhang Imperial College London, UK

* "la Caixa" Banking Foundation cnio stop cancer

Madrid 23 - 25 Sept 2019

Abstract Submission deadline 1 July Application deadline 2 September

Heterogeneity and Evolution in Cancer

Oraanisers

Speakers

Fátima Al-Shahrour Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Arnold Levine The Simons Center for Systems Biology, Institute for Advanced Study, Princeton, US

Raúl Rabadán Columbia Systems Biology, Columbia University, New York, US

Simon Tavaré The Irving Institute of Cancer Dynamics at Columbia University, New York, US

Alexander R. A. Anderson Moffitt Cancer Center and Research Institute, Tampa, US

Niko Beerenwinkel ETH Zürich, Switzerland

Ivana Bozic University of Washington, US

Curtis G. Callan Princeton University. Lewis-Sigler Institute for Integrative Genomics, US

Neal G. Copeland University of Texas MD Anderson Cancer Center, Houston, US

Christina Curtis Stanford University, School of Medicine, Stanford, US

Adolfo Ferrando Institute for Cancer Genetics Columbia University Medical Center, New York, US

Trevor Graham Barts Cancer Institute, London, UK

Benjamin D. Greenbaum Icahn School of Medicine at Mount Sinai, New York, US

Nancy Jenkins University of Texas MD Anderson Cancer Center. Houston, US

Christina Leslie Memorial Sloan Kettering Cancer Center, New York, US

Arnold Levine The Simons Center for Systems Biology, Institute for Advanced Study, Princeton, US

Nuria Lopez-Bigas Institute for Research in Biomedicine, Barcelona, Spain

Scott W. Lowe Memorial Sloan Kettering Cancer Center, New York, US

Guillermina Lozano Univerity of Texas Anderson Cancer Centre, Houston, US

Marta Łuksza Institute for Advanced Study, Princeton US

Florian Markowetz University of Cambridge CRUK Cambridge Institute, UK

FRONTIERS MEETINGS 2019

Memorial Sloan Kettering Cancer Center, New York, US

David Posada School of Biology, University of Vigo, Spain

Columbia University, New York, US

Darrvl Shibata Keck School of Medicine of USC, Los Angeles, US

Andrea Sottoriva The Institute of Cancer Research, London, UK

Doug Winton Cancer Research UK **Cambridge Institute** Cambridge, UK





Dana Pe'er

Carol Prives

Benjamin J. Raphael Lewis-Sigler Institute for Integrative Genomics. Princeton University, US

iť




















CNIO - LA CAIXA BANKING FOUNDATION FRONTIERS MEETINGS | 2019

CNIO "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 20 - 22 May 2019

Structural and molecular biology of the DNA damage response

Centro Nacional de Investigaciones Oncológicas (CNIO) Spanish National Cancer Research Centre Melchor Fernández Almagro, 3 28029 Madrid, Spain www.cnio.es

Coordination and edition: Mercedes Moro, CNIO, Madrid, Spain Production of art and design by Soda Graphics Photographic archive CNIO

This work is subject to copyright. All rights are reserved, whether the whole or part of the materials is concerned, specifically the rights of translation, reproduction on microfilms or in any other way and storage in databanks.

© Fundación CNIO Carlos III, 2019 Printed in Spain

#CFM_DNADamage
@CNIO_Cancer

🕎 @CaixaCiencia

cnio stop cancer









As a non-profit organisation, we would like to thank all those who sponsored this conference. Such contribution helps us to ensure that our conferences will continue to establish the CNIO as a point of reference for the international cancer research community.

#CFM DNADamage

8

@CNIO_Cancer

💟 @CaixaCiencia









